The Early Development of the Marsupialia, with Special Reference to the Native Cat (Dasyurus Viverrinus).

(Contributions to the Embryology of the Marsupialia, IV.)

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INTRODUCTION.

"In mammalian embryology very many surprises are yet in store for us" (Hubrecht, '08).

THE present contribution contains an account of the principal results and conclusions at which I have arrived after a somewhat protracted and much interrupted study of an extensive collection of early developmental stages of Marsupials, ranging from the fertilised egg to the blastocyst in which the two primary germ layers are definitely established. I believe I am now able to give for the first time an account of early Marsupial ontogeny, based on the examination of an adequate material, and both consistent in itself and with what we know of the early development in the other two Mammalian sub-classes. The material at my disposal was obtained during my tenure of office in the University of Sydney, and with the aid of grants from the Royal Society and of a George Heriot Research Fellowship. It represents the proceeds of some eight years' collecting, and comprises a fairly complete series of stages of the native cat (Dasyurus viverrinus), together with a few early stages of other Marsupials, notably Perameles and Macropus.

Dasyurus proved in many ways a convenient subject for embryological purposes. It can readily be trapped in many districts in New South Wales; it lives and breeds fairly well in captivity, and though always somewhat intractable, it can, owing to its size, be easily handled, and so may be subjected

if necessary to daily examination.[1] But it has this great disadvantage, which it apparently shares with other Marsupials, that a very variable period intervenes between coitus and ovulation. As a consequence, the obtaining of any desired cleavage or early blastocyst stage is largely a matter of chance.[2] It is true that the changes which take place in the pouch, in correlation with ovulation and the events connected therewith, do afford in the case of late pregnant females some indication of the stage of development likely to be met with, but these changes are at first of too indefinite a character to be of much service beyond indicating that ovulation may have taken place.

Dasyurus breeds but once a year, the breeding season extending over the winter months—May to August. One remarkable feature in the reproduction of Dasyurus, to which I have directed attention in a previous paper (Hill, '00), may be again referred to here, and that is the fact that there is no correlation between the number of ova shed during ovulation and the accommodation available in the pouch. The normal number of teats present in the latter is six, though the presence of one or two supernumerary teats is not uncommon; the number of ova shed at one period is, as a rule, far in excess of the teat number. I have, for example, several records of the occurrence of from twenty to twenty-five eggs, two of twenty-eight, one of thirty, and one of as many as thirty-five! (twenty-three normal blastocysts and twelve

[1] Perameles, on the other hand, though quite common in many parts of the State, is by no means such a convenient type It is much less easily trapped than Dasyurus, does not live nearly so well in captivity, and is particularly difficult to handle. I have to thank Mr. D. G. Stead, now of the Department of Fisheries, Sydney, for first directing my attention to the breeding habits of Dasyurus, and also for providing me with the first female from which I obtained segmenting eggs.

[2] For example, I obtained unsegmented ova from the uteri, four, five, six, seven and eight days after coitus, 2-celled eggs six and seven days after, 4-celled eggs eleven and eighteen days after. In one case the young were born eight days after the last observed act of coitus, in another sixteen days after, and in yet another twenty days after.

abnormal). There can be little doubt that Dasyurus, like various other Marsupials (e.g. Perameles, Macropus, etc.), has suffered a progressive reduction in the number of young reared, but even making due allowance for that, the excess in production of ova over requirements would still be remarkable enough. Whether this over-production is to be correlated in any way with the occurrence of abnormalities during early development or not, the fact remains that cleavage abnormalities are quite frequently met with in Dasyurus.

Technique.—As fixatives, I have employed for ovaries the fluids of Hermann, Flemming, Ohlmacher, and Zenker; for ova and early blastocysts, Hermann, Flemming, Perenyi, and especially picro-nitro-osmic acid (picro-nitric acid [Mayer] 96 c.c., 1 per cent. osmic acid 2 c.c., glac. acetic acid 2 c.c.); for later blastocysts, the last-named fluid especially, also picro-corrosive-acetic and corrosive-acetic.

To facilitate the handling of ova and early blastocysts during embedding, I found it convenient to attach each specimen separately to a small square of pig's fœtal membrane by means of a dilute solution of photoxylin (1 to 2 per cent.). Orientation of the specimen was then easily effected during final embedding, under the low power of the microscope. The larger blastocysts were double-embedded in photoxylin and paraffin, the cavity of the blastocyst being tensely filled with the photoxylin solution by means of a hypodermic syringe fitted with a fine needle.

For the staining of sections, Heidenhain's iron-hæmatoxylin method proved the most satisfactory, and was almost exclusively employed. Entire portions of the blastocyst wall were stained either with Ehrlich's or Delafield's hæmatoxylin.

I am much indebted to Mr. L. Schaeffer, of the Anatomical Department of the University of Sydney, and to Mr. F. Pittock, of the Zoological Department, University College, for invaluable assistance in the preparation of the photomicrographs reproduced on Plates 1–5, and also to Mr. A. Cronin, of Sydney, and Miss M. Rhodes, for the drawings from their respective pencils reproduced on Plates 6 and 7.

To Miss V. Sheffield I am indebted for the original of fig. 63. To my friend Dr. F. P. Saudes, Sydney, I am indebted for kind help in the revision of certain parts of the manuscript.

Chapter I.—Critical Review of Previous Observations on the Early Development of the Marsupialia.

Apart from the very brief abstract of a short paper on the development of Dasyurus, which I read before Section D of the British Association in 1908 (included in Dr. Ashworth's Report, 'Nature,' vol. lxxviii), our knowledge of the processes of cleavage and germ-layer formation in the Marsupialia is based (1) on the well-known observations of the late Emil Selenka ('86) on the development of the Virginian opossum (Didelphys marsupialis), published in 1886 as Heft 4 of his classical 'Studien'; and (2) on those of W. H. Caldwell ('87) on the uterine ovum, and cleavage process in the native bear (Phascolarctus cinereus).

Selenka's account of the mode of origin of the germ-layers in Didelphys differs widely, as the sequel will show, from my description of the same in Dasyurus. Now Didelphys and Dasyurus are two marsupials, admittedly allied by the closest structural ties, and we should therefore not expect à priori that they would differ fundamentally in the details of their early ontogeny, however much they might diverge in respect of the details of their embryonal nutritional arrangements.

Furthermore, we might reasonably hope, in view of the generally admitted relationships of the Marsupialia, that a knowledge of their early development would aid us in the interpretation of that of Eutheria, or, at least, that their early developmental phenomena would be readily comparable with those of Eutheria. It cannot be said that Selenka's observations realise either of these expectations. "Whichever view is taken of Selenka's description of the opossum," writes Assheton ('98, p. 254), "many obvious difficulties remain for the solution of which no satisfactory suggestion can as yet be offered."

As concerns my own observations, I venture to think it is possible to bring them into line with what we know of the early ontogeny in the other two mammalian sub-classes, and I have attempted to do so in the concluding chapter of this paper, with what success the reader can judge, whilst as regards the divergence between Selenka's results and my own, I am perfectly convinced that the explanation thereof is to be found in the fact that the whole of Selenka's early material was derived from but two pregnant females, and that much of it consequently consisted of eggs which had failed to develop normally. From the one female, killed 5 days after coition, he obtained one egg in the 2-celled stage, one with about twenty cells and nine unfertilised ova. From the second, killed 5 days 8 hours after coition, he obtained "ausser zwei tauben, 14 befruchtete Eier nämlich je ein Ei mit 4, 8, 42, 68 Zellen, eine junge und eine ältere Gastrula mit noch dicker Eiweisschicht und endlich acht auch gleichen Entwickelungsstufe stehende weit grössere Keimblasen, deren Wand noch grösstentheils einschichtig war" ('86, p. 112). Selenka recognised that the last-mentioned blastocyst "die normale Entwickelungsphäse repräsentiren," since he found as a rule that all the embryos from one uterus were in the same developmental stage. Nevertheless he proceeded to describe the segmenting eggs and the two "gastrulæ" which lagged so far behind the blastocysts, as if they were perfectly normal developmental stages. He does, indeed, question whether or not the 42-celled stage is normal, but decides in the affirmative, "denn wenn ich von zwei Zweifelhaften Fällen absehe, so habe ich niemals Eier aus den ersten Tag aufgefunden, welche auf irgend welche Anomalie der Entwickelung hinwiesen." This, however, can hardly be accepted as a satisfactory reason for his conclusion, since apart from the other eggs of the same batch, he had but the two eggs from the other female for comparison, viz. the 2-celled egg (and even that is, in my view, not quite normal), and the 20-celled egg, which is stated to have suffered in preparation. With the exception of the two eggs just mentioned, all the crucial

early stages (ranging from the 4-celled stage to the completed blastocyst), on whose examination Selenka based his account of germ-layer formation in Didelphys, would thus appear to have been derived from a single female.[1] No wonder it is impossible to reconcile his description either with what we know of germ-layer formation in the Prototheria and Eutheria or with my account of the same in Dasyurus.

My own experience with the latter has shown me that no reliance whatever is to be placed on segmenting eggs or blastocysts which exhibit marked retardation in their stage of development as compared with others from the same uterus, and also that batches of eggs or blastocysts in which there is marked variation in the stage of development attained should likewise be rejected. Abnormalities in the process of cleavage and of blastocyst formation are by no means uncommon in Dasyurus, and during the earlier stages of my own work I spent much time and labour on the investigation of just such abnormal material as that on which Selenka, no doubt unwittingly, but I feel bound to add, with an utter disregard for caution, based his account of the early development of Didelphys.

I propose now, before passing to my own observations, to give a short critical account of Selenka's observations, my comments being enclosed in square brackets.

The uterine ovum of Didelphys is enclosed by (1) a relatively thin "granulosamembran," formed by the transformation of a layer of follicular cells [really the shell-membrane, first correctly interpreted by Caldwell ('87) and formed in the Fallopian tube]; (2) a laminated layer of albumen, semitransparent; (3) a zona radiata, not always recognisable [in my experience invariably distinct].

Cleavage begins in the uterus, is holoblastic, and at first equal. A 2-celled stage is figured (Taf. xvii, fig. 3) [not quite normal as regards the relations of the blastomeres], and also a 4-celled stage [normal in appearance except for the

[1] The collection of my own early material of Dasyurus has involved the slaughter of over seven dozen females.

enormous thickness of the albumen layer], in which the four equal-sized blastomeres are radially arranged round a cleavage cavity and are conical in form, their upper ends being more pointed, their lower ends thicker and richer in yolk-material. The nucleus of each is excentric, being situated nearer the upper pole. [This description is applicable word for word to the 4-celled stage of Dasyurus.]

An 8-celled stage (fig. 6) is next described, seven of the blastomeres being equal in size and one being smaller. They are arranged somewhat irregularly in two circles. [This stage I regard as abnormal both in respect of the arrangement of the blastomeres and the occurrence of irregularity amongst them.] Selenka (p. 119) thought it probable that the third cleavage planes cut the first two at right angles and divided each of the first four blastomeres into a smaller ectodermal cell and a larger more granular entodermal, but states that he was unable to establish this owing to the opacity of the albumen-layer. [My observations show that it is the fourth cleavage in Dasyurus, not the third, which is equatorial, unequal, and qualitative, and that even then the cells formed are not ectodermal and entodermal in significance. The albumen is normally never opaque.]

A 20-celled stage is mentioned, but not described, since it suffered in preparation. It is said to have a large entoderm cell in the cleavage cavity. [A statement of very doubtful value, since the blastomeres were admittedly pressed together and probably displaced by the shrunken egg-membranes.]

The next stage described is a spherical "gastrula" (Taf. xvii, figs. 7, 8), composed of forty-two cells with an open "blastopore" at the vegetative pole, a smaller opening at the animal pole, and a large "ur-entoderm" cell in the cleavage-cavity, just inside the "blastopore." The wall of the "gastrula" consists of cells graduated in size; those in the region of the blastopore are the largest and richest in deutoplasm, those at the opposite pole are the smallest and most transparent. [This is a very characteristic stage in the formation of the blastocyst, with which I am quite familar in Dasyurus. Selenka's speci-

men, judging from Dasyurus, is normal as regards the constitution of its wall and the occurrence of an opening at each pole. The lower opening, however, has no blastoporic significance, but, like the upper, owes its presence to the mode of formation of the blastocyst-wall by the spreading of the blastomeres towards the poles of the sphere formed by the egg-envelopes. Selenka's blastopore simply marks the last point of closure. This specimen I hold to be abnormal from the presence of the so-called "urentoderm" cell in its interior. I figure (Pl. 3, fig. 37) a section of a fairly comparable and undoubtedly abnormal blastocyst of Dasyurus in which there is also present in the blastocyst cavity a large free cell. Here this latter is unquestionably a blastomere of the lower hemisphere, which, having failed to divide, has become enclosed by the spreading of its neighbours. Selenka's "urentodermzelle" I regard as a similarly displaced blastomere.]

A 68-celled "gastrula" (figs. 9 and 10) is next described. It is essentially similar to the preceding, only the "blastopore" has closed.

The succeeding stage (fig. 11) is a somewhat older "gastrula," in which gastrulation is said to be still in progress, since over the lower pole, in the region of the now closed blastopore, it is no longer possible to say which cells belong to the ectoderm, which to the entoderm. The latter layer is described as being several cells thick in the blastoporic region, and as in course of spreading round inside the ectodermal wall of the "gastrula" towards the upper or animal pole. [This specimen is undoubtedly abnormal, at all events there is no comparable stage in Dasyurus. It is difficult to obtain a clear idea of Selenka's conception of the mode of origin of the germ-layers, but he evidently held (cf. pp. 116 and 119) that the large yolk-rich cells of the lower ("blastoporic") pole constitute the anlage of the entoderm, and that they become inturned at the "blastopore" and proliferate to form the definitive entoderm, which then gradually extends round to the animal pole, in contact with the inner surface of the wall of the gastrula, that wall forming the ectoderm. He appa-

rently did not regard the "urentodermzelle" as the sole progenitor of the entoderm, but simply as an entoderm-cell precociously inturned from the "blastoporic" margin.

This view of Selenka, however, lands us in the predicament of having to regard the embryonal area as differentiating over the vegetative hemisphere, since in the next stage the "blastopore" is described as being situated excentrically in that area. Either Selenka's determination of the poles in the 42-celled blastocyst is wrong, or the entoderm does not originate as he describes it. My own observations force me to accept the latter alternative. In his paper Selenka gets over the difficulty very easily by altering the orientation of his figures. On Taf. xvii, the figures of sections of blastocysts are so placed that the "blastopore" is below, next the bottom of the plate. These figures I hold to be correctly orientated. On Taf. xviii, the figures are inverted, so that the "blastopore" is above; as the result the animal pole of fig. 11, Taf. xvii, becomes the vegetative pole of the stage next described (fig. 2, Taf. xviii).]

The stage just referred to, described as an "eiförmige gastrula," is represented in a drawing made from the fresh specimen as lying quite free in a large perivitelline space enclosed by a very thick layer of albumen, outside which is the "granulosa-membran." In section (fig. 2) a mass of entoderm is seen to reach the surface at one pole (marked *bl.*) uppermost in the figure, whilst other entodermal cells are shown spreading from this towards the lower pole. The ectoderm of the wall is represented as composed of definitely cubical cells. [The presence of a large perivitelline space, by itself stamps this specimen as not normal. The sectional figure must be schematic.]

The last of Selenka's early stages to which reference need be made here is formed by eight "gastrulæ" (blastocysts), reckoned as ten hours after the commencement of cleavage [a reckoning I consider of no value] (Taf. xviii, figs. 3 and 4). The embryonal area is now distinguishable by the larger size of its ectodermal cells. The entoderm is unilaminar, and has

extended beyond the limits of the embryonal area. The position of the "blastopore" is said to be marked in all by a mass of coagulum attached to the wall, and in three by a definite opening as well. It is situated excentrically in the embryonal area. [Except for the "blastopore" and the presence of a thick layer of albumen, this blastocyst stage is quite comparable with the corresponding one in Dasyurus; the latter, however, is considerably larger. Of Selenka's early material, I think it is these blastocysts alone which had any chance of giving origin to normal embryos.]

W. H. Caldwell, who, as Balfour student, visited Australia in 1883–4, obtained a very rich collection of early marsupial material, of which, unfortunately, no adequate account has ever been published. He gave, however, in his introductory paper on the 'Embryology of the Monotremata and Marsupialia' ('87), an account of the structure of the ovum, both ovarian and uterine, in Phascolarctus, and he showed that the ovum during its passage down the Fallopian tube becomes enclosed outside the albumen layer in "a thin transparent membrane, ·0015 mm. thick," which he homologised with the shell-membrane of the monotreme egg. This important discovery of the existence of a shell-membrane in the Marsupialia I can fully confirm. I am, however, unable to accept his interpretation of the internal structure of the ovum of Phascolarctus, or his remarkable statement that cleavage in that form is of the meroblastic type. Cleavage is not described in detail, nor is any account given of the mode of origin of the germ-layers.

Chapter II.—The Ovum of Dasyurus.

1. Structure of the Ovarian Ovum.

The full-grown ovarian ovum of Dasyurus (Pl. 1, fig. 1) appears as a rounded, or more usually, ovalish cell, the diameter of which varies in section in ten eggs measured from ·28 × ·126 mm. to ·27 × ·26 mm. (average, ·24 mm.), and is therefore large relatively to the ova of Eutheria. It

is enclosed by a thin, but very definite refractive membrane or zona (vitelline membrane of Caldwell) of an approximate thickness of ·002 mm. (fig. 1, *z.p.*), on which the cells of the discus proligerus (fig. 1, *d.p.*) directly abut, a differentiated corona radiata and syncytial layer being absent. It appears to be identical in its relations and optical characters with the membrane investing the monotreme ovum, and never shows in section any trace of radial striations (though I believe I have detected an extremely faint appearance of such in the fresh zona), or of the extension into it of protoplasmic processes from the adjacent cells of the discus proligerus, such as Caldwell figures in the case of the ovum of Phascolarctus (cf. his Pl. 29, fig. 5). Within the zona the peripheral cytoplasm of the ovum is differentiated to form an exceedingly thin but distinct bounding layer or egg-membrane (vitelline membrane, sensu stricto).

The cytoplasmic body of the ovum exhibits a very obvious and striking differentiation into two regions in correspondence with the presence in it of two definitely localised varieties of deutoplasmic material, respectively granular and fluid. Peripherally it consists of a relatively narrow cytoplasmic zone of practically uniform width, dense and finely granular in appearance owing to the presence in it of numerous particles of deutoplasmic nature. This we may distinguish as the formative zone (fig. 1, *f.z.*). In it lies embedded the large vesicular nucleus (about ·06 × ·03 mm. in diam.). Centrally and forming the main bulk of the ovum is a mass of greatly vacuolated cytoplasm presenting the appearance of a clear wide-meshed reticulum. Its framework is coarser peripherally where it passes over without definite limit into the formative zone, with which it is structurally identical, but much finer and wider-meshed centrally, so fine, indeed, that it almost invariably breaks down under the action of fixatives, and appears in sections as an irregular space, perhaps crossed by a few fine interlacing strands (fig. 1, *d.z.*). The meshes of this reticulum are occupied by a clear fluid which must be held to constitute the central deutoplasm of the egg. We

may accordingly designate this central reticular area as the deutoplasmic zone.

If we pass now from the full-grown to the ripe ovarian ovum (Pl. 1, figs. 2 and 3), i. e. an ovum in which either the first polar spindle has appeared or the first polar body has already been separated off, it at once becomes evident that important changes have occurred in the disposition and relative proportions of the two constituent regions of the egg-cytoplasm. The full-grown ovum is of the centrolecithal type, the central deutoplasmic zone forming its main bulk and being completely surrounded by the thin formative zone. The ripe ovum, on the other hand, exhibits an obvious and unmistakable polarity, and is of the telolecithal type, as the following facts show. The cytoplasmic body evidently consists of the same two regions as form that of the full-grown ovum, but here the dense formative region now forms its main bulk, and no longer surrounds the clear deutoplasmic region as a uniform peripheral layer. It has not only increased considerably in amount as compared with that of the full-grown egg, and at the expense apparently of the more peripheral coarser portion of the deutoplasmic zone, but it has undergone polar segregation, with the result that it now occupies rather more than one hemisphere of the egg as a dense finely granular mass, with vacuoles of varying size sparsely scattered through it (figs. 2 and 3, *f.z.*). It accordingly defines one of the ovular poles. The opposite pole is just as markedly characterised by the presence immediately below it of a more or less rounded clear mass, eccentrically situated, and composed of an extremely fine cytoplasmic reticulum with wide fluid-filled meshes. It is completely surrounded by formative cytoplasm (though over the polar region the enclosing layer is so extremely thin that it here almost reaches the surface), and its cytoplasmic framework is perfectly continuous with the same, the line of junction of the two being abrupt and well defined. So delicate, however, is this framework that it breaks down more or less completely under the action of fixatives of such

excellence even as the fluids of Flemming and Hermann, and thus in sections usually all that represent it are a few irregular cytoplasmic strands crossing a large, sharply defined clear space (figs. 2 and 3, *d.z.*). The mass in question has thus all the characters of the deutoplasmic zone of the full-grown ovum, and it must undoubtedly be held to represent the central portion of that which has not been utilised in the upbuilding of the formative cytoplasm, and which has been forced to take up an excentric position immediately below the polar region of one hemisphere, owing to the increase of the formative cytoplasm and its segregation in the other hemisphere.

The ripe ovum of Dasyurus thus possesses a polarity which in its way is equally as striking as that of the Monotreme egg. Towards the one pole the main mass of the ovum is composed of dense, slightly vacuolated formative cytoplasm, in which the polar spindle is situated peripherally, but nearer the equator than the formative pole. Toward the opposite pole and practically reaching the surface is a rounded mass of greatly vacuolated deutoplasmic cytoplasm. Roughly, the formative cytoplasm constitutes about two-thirds of the bulk of the ripe egg, the deutoplasmic the remaining third. Such being the structure of the ripe ovarian egg, if we classify it at all, we must place it, it seems to me, with eggs of the telolecithal type. My view of the significance of this marked polar differentiation of the constituent materials of the ripe ovum of Dasyurus I shall presently indicate. Meantime I would lay special emphasis on the fact that the eccentric mass of deutoplasmic cytoplasm represents material, surplus deutoplasmic material which has not been utilised in the upbuilding of the formative cytoplasm.

The fact of the occurrence in the Eutherian ovum of a polar differentiation of its constituent materials is now definitely established, thanks especially to the valuable researches of Prof. O. Van der Stricht and his pupils—H. Lams and the late J. Doorme. In this connection I wish to refer here in some detail to the extremely interesting obser-

vations of Van der Stricht ['03, '05] on the structure and polarity of the ovum of the bat (Vesperugo noctula), since these observations are in essential agreement with my own on the ovum of Dasyurus, and enable me to affirm that the polar differentiation herein recorded for the first time for the Marsupial ovum is attained as the result of vitellogenetic processes, which essentially correspond with those of the ovum of the bat. Van der Stricht, as is well known, has made a special study of the process of vitellogenesis in the Eutherian ovum, and is, indeed, at the present time the foremost authority on this particular subject, so that his views are worthy of all respect.

Study of the oöcyte of Vesperugo during the period of growth shows, according to Van der Stricht, that "a un moment donné du développement du jeune œuf, les boyaux et amas vitellogènes [derived, according to him, from 'une couche vitellogène, mitochondriale,' present in the young oöcyte in the first stage of growth] disparaissent au profit du vitellus, dont la structure pseudo-alvéolaire s'accentue graduellement." The full-grown oöcyte at the stage just prior to the appearance of the first polar spindle is characterised by the presence of this "pseudo-alveolar structure" throughout the extent of its cytoplasmic body. The alveoli or vacuoles are of variable size, are filled by a clear liquid, and "correspondent incontestablement au deutoplasma de l'œuf. A ce stade du développement de l'oöcyte, ce vitellus nutritif, auquel s'ajoutent bientôt des granulations graisseuses, est répandu uniformément dans toutes les profondeurs du cytoplasme. Nulle part on ne constate une zone deutoplasmique distincte d'une zone de vitellus plastique." In Dasyurus the stage in vitellogenesis which almost exactly corresponds with that of the full-grown oöcyte of Vesperugo just described is seen in oöcytes not quite full-grown. In fig. 4 is shown an oöcyte of Dasyurus (·26 × ·20 mm. in diameter), in which the same pseudo-alveolar structure as described by Van der Stricht for the Vesperugo oöcyte is perfectly distinct. Here, however, fatty particles are not

apparent, and the peripheral portion of the cytoplasm tends to be free from vacuoles. In Dasyurus the formation of these deutoplasmic vacuoles begins in oöcytes about ·2 mm. or less in diameter. This characteristic "pseudo-alveolar" stage is followed in both Vesperugo and Dasyurus by one in which there is recognisable in the cytoplasmic body of the ovum a differentiation into a dense peripheral zone and a central vacuolated area. In Vesperugo this stage is attained about the time of appearance of the first polar spindle, whilst in Dasyurus it is attained somewhat earlier, always prior to the formation of the latter. So close is the agreement between the two forms that Van der Stricht's description of the bat's egg at the time of appearance of the first polar spindle might equally well be applied to the full-grown ovum of Dasyurus. He writes ['03, p. 43]: "Vers l'époque de l'apparition du premier fuseau de maturation, le vitellus prend un autre aspect. La partie centrale deutoplasmique conserve une structure pseudo-alvéolaire, mais dans le voisinage immédiat du premier fuseau et dans toute l'étendue de la couche périphérique du protoplasme, apparaît une mince zone de vitellus compact et dense, plus ou moins homògene où les vésicules claires font défaut. . . . A ce moment, on distingue dans l'oöcyte de V. noctula une zone centrale très étendue, riche en deutoplasme et une zone corticale très mince, riche en vitellus plastique." This centro-lecithal phase, as we may term it, is followed in Vesperugo during fertilisation and the separation of the second polar body by a telolecithal phase characterised by a distinct polarity. "La zone de vitellus plastique s'épaissit encore, mais surtout à un pôle de l'œuf, à celui opposé au pôle où se détachent les deux globules polaires. Ce pôle, où s'accumule graduellement le vitellus formateur, mérite le nom de pôle animal. Il est opposé au pôle d'expulsion des globules polaires, vers lequel est refoulé le deutoplasme, et qui se comporte désormais comme le pôle végétatif. Pendant que les deux pronucléus mâle et femelle se forment, le vitellus plastique augmente graduellement en abondance au pôle

animal, tandis qu'il diminue au pôle végétatif, et le deutoplasme, parsemé d'un plus grand nombre de boules graisseuses, constitue une masse sphérique excentrique, voisine des deux globules polaires" (Van der Stricht, '03, pp. 44–45). It is evident, then, that the fertilised ovum of Vesperugo exhibits a polarity comparable with that of the ripe ovarian ovum of Dasyurus, and that the vitellogenetic processes in the ova of these two widely separated forms proceed along lines almost identical, at all events so far as their broad outlines are concerned. In both we find during growth a progressive vacuolisation of the egg-cytoplasm consequent on the elaboration of a deutoplasmic fluid. In both, the "pseudo-alveolar" condition so engendered is followed by one in which there is recognisable a differentiation into a peripheral "formative" zone rich in deutoplasmic granules, and a central "deutoplasmic" zone rich in fluid yolk, and finally in both there occurs a segregation of the granular "formative" and fluid yolk-constituents to opposite regions of the egg, with resulting attainment of a definite polarity. In view of the close general agreement in the vitellogenetic processes, and in the constitution of the ova in Vesperugo and Dasyurus, it might be expected that the poles would accurately correspond, but such is not the case if Van der Stricht's determination of the poles in the ovum of Vesperugo is correct. In the latter, according to Van der Stricht, the deutoplasm is located at that pole from which the polar bodies are given off; at the opposite pole the "plastic" vitellus accumulates, and close to it the two pronuclei unite and the first cleavage spindle is formed. Accordingly Van der Stricht concludes that "le premier pôle correspond au pôle végétatif, le second au pôle animal des œufs à deutoplasme polaire (O. Hertwig)." In Dasyurus, on the other hand, I am perfectly convinced (and adequate reason for my conviction will be forthcoming in the course of my description of the processes of cleavage and germ-layer formation) that the pole of the ripe ovum in relation to the mass of deutoplasmic cytoplasm is not the vegetative pole, but represents morphologically the upper or

animal pole of the egg, the opposite pole in relation to which the formative cytoplasm is situated being the lower or vegetative. The deutoplasmic cytoplasm thus lies in the upper hemisphere, whilst the formative cytoplasm occupies the lower. If Van der Stricht's determination of the poles of the ovum of Vesperugo be accepted, then we must conclude that the poles of the Dasyurus ovum are exactly reversed as compared with those of the bat's egg. In this connection it may be recalled that Lams and Doorme ['07] have demonstrated the occurrence in Cavia of an actual reversal of the original polarity of the ovum, prior to the beginning of cleavage. These facts may well give us pause before we proceed to attach other than a purely secondary significance to the exact location of the formative and deutoplasmic constituents in the Metatherian and Eutherian ovum. But besides this apparent difference in the location of the deutoplasmic constituents of the ova of Dasyurus and Vesperugo, there exists yet another which concerns the fate of these constituents in the respective eggs. In Vesperugo, Van der Stricht shows that the "deutoplasm" remains an integral part of the egg, and retains its polar distribution in the blastomeres up to at least the 4-celled stage.[1] In Dasyurus, on the other hand, the fate of the deutoplasmic mass is a very different, and, indeed, a very remarkable one. It does not remain an integral part of the segmenting egg as in Vesperugo, but prior to the completion of the first cleavage furrow it becomes bodily separated off, apparently by a process of abstriction, from the formative cytoplasm as a clear rounded mass which takes no further direct part in the developmental processes. As soon as its elimination is effected, the remainder of the cytoplasmic body of the ovum, formed of the formative cytoplasm alone, divides into the first two equal-sized blastomeres, the first cleavage plane being coincident with the polar diameter and at right angles to the plane of separation of the deutoplasmic mass, or "yolk-body" as we may term it (Pl. 2, figs. 14–16, 19, *y.b.*), so that it is this formative zone of the

[1] Vide, however, "Addendum" (p. 121).

ovum which is alone concerned in the production of the embryo and its fœtal membranes.

We have but to recall the conclusion already reached that the clear vacuolated zone at the upper pole of the ripe ovum of Dasyurus consists of surplus material, mainly in the form of fluid of deutoplasmic nature which has not been utilised in the upbuilding of the formative cytoplasm, and the significance of this remarkable and, so far as the Mammalian ovum is concerned, absolutely unique occurrence becomes at once manifest.[1] We have to do here with an actual elimination of surplus deutoplasmic material by the Marsupial ovum—a phenomenon only paralleled elsewhere, so far as I am aware, and even then but distantly, by the curious temporary separation of the so-called yolk-lobe which occurs during the cleavage of the yolk-laden eggs of certain Molluscs (Nassa, Ilyanassa, Modiolaria, Aplysia, Dentalium) and Annelids (Myzostoma, Chætopterus). In these forms cleavage of the ovum into the first two blastomeres is accompanied by the separation of a portion of the ovular substance in the form of a non-nucleated mass or so-called yolk-lobe. This latter, which has been shown to be connected with the formation of determined organ-anlagen, reunites with one of the two blastomeres, and then the same process of abstriction and reunion recurs at the second cleavage.[2] We have here evidently a purely adaptive phenomenon, the object of which no doubt is to permit of the total cleavage of the yolk-laden ovum on what are presumably the old ancestral lines, and I believe a comparable explanation will be found applicable to the elimination of surplus yolk-material by the Marsupial ovum.

As regards the significance of the occurrence of the deutoplasmic zone in the ovum of Dasyurus, holding the views that I do as to the phylogeny of the Marsupialia (viz. that the Metatheria and Eutheria are the divergent branches of a

[1] Vide "Addendum" (p. 121), in which reference is made to the discovery by Prof. Van der Stricht of the elimination of deutoplasm in the ovum of Vesperugo.

[2] Cf. Korschelt u. Heider, 'Lehrbuch d. vergl. Entwicklungsgeschichte,' Lief. 3, p. 107, 1909.

common stock, itself of Prototherian derivation), and bearing in mind the occurrence of an undoubted representative of the shell round the Marsupial ovum, I venture to see in the fluid-material of the deutoplasmic zone the partial and vestigial equivalent of the yolk-mass of the monotreme egg. In other words, I would regard the deutoplasmic fluid as the product of an abortive attempt at the formation of such a solid yolk-mass. The objection will no doubt be forthcoming that this interpretation cannot possibly be correct since the supposed equivalent of the yolk-mass in the Dasyure ovum is located, on my own showing, at the wrong pole—at the upper instead of at the lower. But its precise location does not seem to me to be a matter to which we need attach any great importance, since it has doubtless been adaptively determined in correlation with the special character of the cleavage process.

The belief that the minute yolk-poor ovum of the Eutheria is no pure primarily holoblastic one, but that it has only secondarily arrived at the total type of cleavage as the result of the all but complete loss of the yolk ancestrally present in it, consequent on the substitution of the intra-uterine mode of development for the old oviparous habit, is now widely held amongst Mammalian embryologists. Hubrecht, however, is an exception, wedded as he is to a belief in the direct derivation of the Eutheria from Protetrapodous ancestors with yolk-poor, holoblastic eggs. Whether the interpretation I have put forward, viz. that the non-formative or deutoplasmic zone of the Dasyure ovum is the reduced and partial equivalent of the yolk-mass of the Monotreme egg, be accepted or not, I venture to think that my discovery of an actual elimination of deutoplasmic material by the Marsupial ovum affords a striking confirmation of the truth of the prevailing conception as to the phylogeny of the Eutherian ovum, and I further venture to think that the facts I have brought forward in the preceding pages justify us in regarding the ripe ovarian ovum of Dasyurus as being potentially of the yolk-laden, telolecithal type, and the uterine ovum, by bodily casting out the superfluous part of its deutoplasm, as becoming at the same time

secondarily homolecithal and secondarily holoblastic. The Marsupial ovum presents itself to my mind as the victim of tendencies conditioned by its ancestry, and in particular it appears as if its inherited tendency to elaborate yolk had not yet been brought into accurate correlation with the other changes (reduction in size, intra-uterine development), which it has undergone in the course of phylogeny. As the consequence it manufactures more yolk than it can utilise, and so finds itself under the necessity of getting rid of the surplus. Whether or not a comparable elimination of deutoplasmic material occurs in the ova of other Marsupials, future investigation must decide. I should be quite prepared to find variation in this regard, correlated perhaps with the size of the egg. In the case of Phascolarctus, Caldwell gives the diameter of the ovum as ·17 mm., and his figure of a (horizontal ?) section of the uterine ovum (here produced as text-fig. 1, p. 27) shows a differentiation of the cytoplasmic body of that into vacuolated and granular zones quite comparable with that of the Dasyure ovum. From the few measurements of ova of other marsupials that I have been able to make, it would appear that the ovum of Trichosurus approximates in size to that of Dasyurus, whilst that of Perameles and probably also that of Macropus are smaller. From Selenka's figure I have calculated that the ovum of Didelphys measures about ·13 × ·12 mm. in diameter. In the smaller ova it is quite likely that yolk-formation may not proceed so far as in the relatively large ovum of Dasyurus.

2. Maturation and Ovulation.

The details of the maturation process have not been fully worked out, owing to lack of material. As in the Eutheria (Sobotta, Van der Stricht, Lams and Doorme, and others), the first polar body is separated off in the ovary, the second apparently in the upper part of the Fallopian tube where entrance of the sperm takes place. The first polar figure (late anaphase observed, fig. 5) lies in the formative cyto-

plasm, close below and at right angles to the zona. Its exact site is subject to some slight variation, and is best described as adjacent to the equatorial region of the egg, sometimes nearer the lower pole, more usually, perhaps, nearer the upper. Centrosomes and polar radiations were not observed. The heterotypical chromosomes (gemini) have the form of somewhat irregular, more or less angular granules. I have not been able to determine their number. The figure is barrel-shaped, and almost as broad as long, measuring ·015 × ·013 mm. The first polar body (fig. 6, $p.b^1$.) is small relatively to the size of the egg, its diameters varying round ·03 × ·01 mm., and its shape is that of a flattened bi-convex disc. In uterine eggs there is some evidence pointing to the probability of its having undergone division.

The second polar spindle (figs. 3 and 7) lies immediately subjacent to the first polar body in the fully ripe ovarian ovum. It is shorter than the first, measuring ·013 mm., and much narrower. The second polar body measures about ·015 × ·01 mm. in diameter, and is thus smaller than the first. I have only seen the second polar body in uterine ova, and therefore can only presume that it is separated off in the upper part of the Fallopian tube, subsequently to the penetration of the sperm, as in Eutheria.

Ovulation takes place irrespective of whether copulation has occurred or not, and it is a fact worthy of record that, even if the ova be not fertilised, the pouch and mammary glands undergo the same series of growth changes as are characteristic of, at all events, the earlier stages of normal pregnancy.

The follicular cells of the discus proligerus investing the ovum are already in the ripe follicle in a state of disruption, and I believe they separate completely from the ovum at the moment of dehiscence, so that, except for the zona, the ova are quite naked when they enter the tube. I have no evidence of the existence outside the zona of a layer of proalbumen such as Caldwell describes round the ovum of Phascolarctus. Apparently the ova are shed almost simultaneously, and they

must pass with considerable rapidity down the tubes to the uteri where cleavage begins, for I have only once found a tubal ovum, and that one had evidently been retarded for some reason, and was polyspermic.

3. The Secondary Egg-membranes: Albumen and Shell-membrane.

During the passage of the ovum down the tube it is fertilised, and becomes enclosed externally to the zona by two secondary layers formed as secretions by the cells of the oviducal lining. First of all, the ovum becomes surrounded by a transparent to semi-transparent laminated layer of albumen, ·015 to ·022 mm. in thickness, composed of numerous very delicate concentric lamellæ, and having, normally, numbers of sperms imbedded in it (figs. 8–11, *alb.*, *sp.*). Then outside the albumen layer there is laid down a definite, but at first very thin, double-contoured membrane (figs. 8 and 10, *s.m.*), which, following Caldwell, I have no hesitation in homologising with the shell-membrane of the Monotreme egg. Caldwell in 1887 described and figured a definite membrane enclosing the uterine ovum of Phascolarctus, externally to, and quite distinct from the albumen, which he interpreted as the representative of the shell-membrane of the Monotremata, but owing apparently to the fact that Selenka altogether failed to recognise its true nature in Didelphys, since he regarded it as a derivative of the follicular epithelium, and termed it the "granulosa-membran," this highly significant discovery of Caldwell has been largely ignored. Such a membrane is constantly present and easily recognisable in all the Marsupials (Dasyurus, Perameles, Trichosurus, Macropus, Petrogale, Phascologale, Acrobates, Phascolarctus, Bettongia), of which I have had the opportunity of studying early developmental stages. It is laid down in the Fallopian tube, is perfectly distinct from the albumen, and increases in thickness in the uterus, and if it has not the significance which Caldwell has suggested, then I must leave it to those

who decline to accept Caldwell's interpretation to put forward an alternative one, since I am unable to do so.

The shell-membrane of Dasyurus (Pl. 1, figs. 8–11; Pl. 2, figs. 17, 18, *s.m.*) is a transparent, perfectly homogeneous layer, highly refractive in character and of a faint yellowish tint. When fully formed it possesses firm, resistant properties, recalling those of chitin, and is doubtless composed of a keratin base. It is distinguishable at once from the albumen by its optical characters and staining reactions, so that there is not the slightest justification for the supposition that it may represent simply the specially differentiated outermost portion of that layer. In ova which have just passed into the uterus (fig. 10) the shell-membrane is extremely delicate, its thickness being only about ·0016 mm., but even before cleavage begins it has increased to ·002 mm. (fig. 12); in the 2-celled stage (fig. 18) it has reached ·005 mm., in the 4-celled stage (fig. 22) ·0072 mm., whilst in the 16-celled stage (figs. 24–26) it has practically attained its maximum thickness, viz., ·0075–·008 mm. Caldwell's measurements in the case of Phascolarctus agree closely with the above (shell of unsegmented ovum from the uterus, ·0015 mm. thick, that of the ·3 mm. "ovum," ·01 mm.). Its presence renders the thorough penetration of ova and early blastocysts with paraffin a capricious and frequently troublesome operation, and its resistant shell-like nature becomes only too obvious in the process of section-cutting, since it cracks with the utmost readiness (cf. Pl. 3, figs. 32, 37).

The occurrence of a shell-membrane round the Marsupial ovum is a feature of considerable phyletic significance, as I need hardly point out. It shows us that the ancestors of the Metatheria must have been oviparous, or must themselves have come from an oviparous stock, which there is no valid reason for supposing was other than Prototherian in its characters. It also renders untenable the views of Hubrecht to the effect that the Metatheria are the descendants of Eutheria, whilst the Eutheria themselves have been directly derived from some presumed viviparous group of hypothetical Prote-

trapods, unless we are to suppose that the Metatheria are even now on the way to acquire secondarily the oviparous habit, much in the same way as the Monotremes, according to Hubrecht, have long since succeeded in doing.

The occurrence of a shell-membrane round the Marsupial ovum has also an important ontogenetic significance in relation to the mode of formation of the blastocyst, as I shall endeavour presently to show.

4. The Uterine Ovum.

The unsegmented ovum from the uterus (figs. 8–13) consists of the following parts:

(1) The shell-membrane externally, ·0016–·002 mm. in thickness.

(2) The laminated layer of albumen, ·015–·022 mm. or more in thickness.

(3) The zona, about ·0016 mm. in thickness.

(4) The perivitelline space, between the zona and the ovum, occupied by a clear fluid which coagulates under the action of certain fixatives, e. g. Hermann's fluid (fig. 11, *p.s.*), and which has diffused in from the uterus. The minute polar bodies lie in this space, usually nearer the upper pole than the lower.

(5) The ovum proper.

The entire egg is spherical in form, and varies in diameter in the fresh state from about ·3 mm. to ·36 mm. (average about ·32 mm.).

The ovum itself is ovoidal, its polar diameter always slightly exceeding the equatorial. Its average diametrical measurements in the fresh state run about ·25 × ·24 mm., though I have records of ova measuring as much as ·3 × ·29 mm., and I find that there is an undoubted slight variation in the size of the ova of even one and the same batch, as well as in those from different females.

The uterine ovum exhibits the same marked polarity as

characterises the ripe ovarian ovum (the upper pole being marked by the vacuolated deutoplasmic zone (figs. 8–11, *d.z.*), and so far as its cytoplasmic body is concerned it shows no essential difference from that.

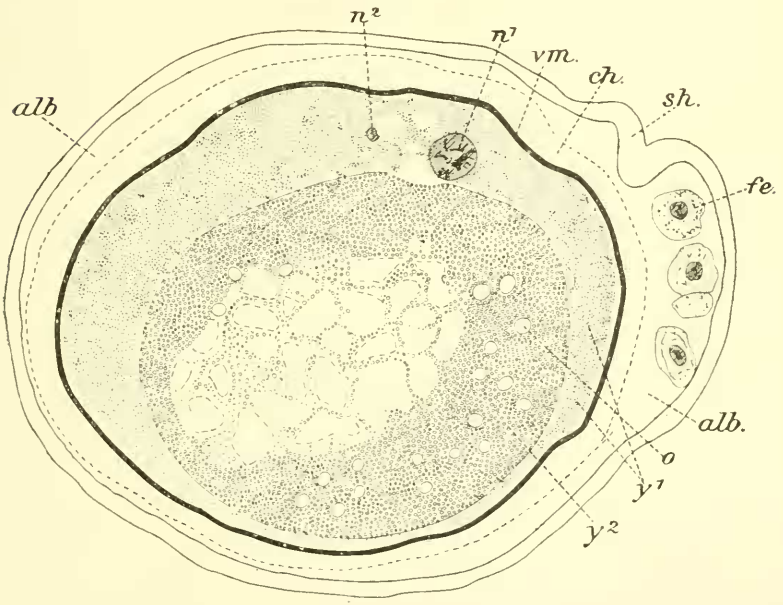
Examined fresh in normal salt solution, the formative cytoplasm forming the bulk of the ovum appears dense, finely granular, and of a very faint lightish-brown tint, its opacity being such that the two pronuclei situated in its central region can just be made out. In section, this central region is distinguishable from the peripheral zone by its uniform, more finely granular character and by the absence of the fluid-filled vacuolar spaces which are generally present in the latter figs. 10 and 12). The deutoplasmic zone at the upper pole, which is only partially visible in the entire egg owing to the way in which it is enclosed by the formative cytoplasm (figs. 8, 9, *d.z.*), presents a characteristically clear or semi-transparent vacuolated appearance in the fresh state, but may have embedded in it a small dense mass (fig. 8, cf. also figs. 11 and 14), evidently formed by the transformation of a portion of its fluid constitutent into the solid state, and so to be regarded as comparable with a bit of formative cytoplasm.

In most of the unsegmented uterine ova at my disposal the male and female pronuclei have attained approximately the same size and lie in proximity in the central more homogeneous region of the formative cytoplasm (figs. 10–12). The transformation of the sperm-head into the male pronucleus probably takes place during the passage of the ovum down the tube, and was not observed, and I am as yet uncertain whether the pronuclei unite to form a single cleavage nucleus or give origin directly to the chromosomes of the first cleavage figure.

Caldwell figures ('87, Pl. 30, fig. 5) a section through the uterine ovum of Phascolarctus which I reproduce here as Text-fig. 1, in order to facilitate comparison with my figs. 11 and 12, with which it shows an essential agreement, apart from the presence of follicular cells in the albumen which I have never observed in Dasyurus, and making allowance for the

difference in sectional plane. The figure is stated to represent "the seventeenth section of a vertical longitudinal series of thirty-five sections through the segmenting ovum, containing two nuclei, taken from the uterus and measuring ·17 mm. in diameter." Caldwell has, I think, fallen into several errors in his interpretation of the structural features seen in this

Text-fig. 1.



Section of uterine ovum of Phascolarctus cinereus.
(After Caldwell.)

figure. In the first place, the sectional plane appears to me not to be vertical as in my own figs. 11 and 12, but horizontal, and to have passed through the lower portion of the deutoplasmic zone, shown in the figure as a central markedly vacuolated area. Then there is no evidence to be derived from the figure in support of the description of the ovum as segmenting. The part inside the zona (*vm.*) labelled y^1 and described as "protoplasm with finest yolk-granules," I would

interpret simply as coagulum in the perivitelline space, whilst the so-called "segmentation nuclei" (n_1, n_2) situated in it are probably the polar bodies or their derivatives. The part labelled y_2, and designated "white yolk," I would regard as the ovum itself. It exhibits an obvious differentiation into a central vacuolated area and a peripheral, dense, granular zone with scattered vacuoles, and I think there can be little doubt but that the former corresponds to the deutoplasmic zone of the Dasyure ovum, the latter to the formative zone. It is these errors of interpretation apparently which misled Caldwell into making the statement, now widely quoted in the text-books, that cleavage in Phascolarctus is of the meroblastic type.

Chapter III.—Cleavage and Formation of the Blastocyst.

1. Cleavage.

Cleavage begins in the uterus as in Didelphys, Phascolarctus, and no doubt Marsupials in general. The first externally visible step towards it consists, as already described, in the elimination by abstriction of the deutoplasmic zone at the upper pole. The yolk-body so formed appears as a definitely limited, clear, rounded mass which lies in contact with the slightly concave upper surface of the formative remainder of the ovum. It is quite colourless and transparent except for the frequent occurrence in it of a small, more or less irregular opaque mass, representing probably a condensation product of its fluid material (cf. Pl. , figs. 8, 14, *y.b.*). Consisting as it does of a very delicate cytoplasmic reticulum with fluid-filled meshes it is extremely fragile, and is seen to advantage only in fresh material (figs. 14 and 19, *y.b.*). It takes no direct part in the later developmental processes, though during the formation of the blastocyst it becomes enclosed in the blastocyst cavity and finally undergoes disintegration therein, its substance becoming added to the fluid which fills the same, so that it may be said, in this indirect way, to fulfil, after all, its original nutritional destiny. Separa-

tion of the yolk-body is rapidly followed by the completion of the division of the formative remainder of the ovum into the first two blastomeres, the plane of division being coincident with the polar diameter or egg-axis and at right angles to the plane of separation of the yolk-body (Pl. 2, fig. 14). I obtained relatively little material between the stage of the unsegmented ovum with two equal-sized pronuclei seen in fig. 12 and the 2-celled stage (fig. 14), both of which are well represented in my material, so that it would appear that the separation of the yolk-body and the division of the formative remainder of the ovum are effected with considerable rapidity. Fig. 13 shows, however, a section of an unsegmented ovum in which the chromosomes of the metaphase of the first cleavage figure are visible in the central region of the formative cytoplasm, but situated, it is worthy of note, nearer the future upper pole than the lower pole. The deutoplasmic zone (*d.z.*) still forms an integral part of the egg, and there is no sign of commencing abstriction. I have also sections of ova in a still more advanced stage of the first cleavage, in which the daughter-nuclei have but recently been constituted and are still quite minute, and the cleavage furrow is well marked on the surface of the egg. In these ova the yolk-body is already separated, so that we may conclude with a fair degree of certainty that its elimination about coincides with the first appearance of the cleavage furrow.

Figs. 14–16 show the 2-celled stage, respectively in side, lower polar, and end views. The blastomeres are of approximately equal size and otherwise quite similar. Selenka also found the same to be the case in Didelphys, though in the single specimen of the 2-celled stage he had for examination (Taf. xvii, fig. 3) the blastomeres are displaced and somewhat shrunken. Each blastomere has much the shape of a hemisphere from which a wedge-shaped segment has been sliced off, a form readily accounted for when we take account of the effect of the elimination of the deutoplasmic zone. After that event, the formative remainder of the ovum has the form of a sphere from which a somewhat bi-convex lens-

shaped piece has been gouged out at the upper pole. Consequently, when it divides along its polar diameter, the resulting blastomeres will have the form of hemispheres with obliquely truncated upper surfaces or ends, which will be proportionately thicker than the lower ends. In correlation therewith we find the nucleus of each blastomere situated slightly excentrically, rather nearer the upper than the lower pole (fig. 18). The rounded yolk-body lies partly enclosed between the upper truncated surfaces of the blastomeres.

Two-celled eggs are shown in vertical section in figs. 17 and 18. The cytoplasm of the blastomeres exhibits a well-marked differentiation into two zones corresponding to that already seen in the formative cytoplasm of the unsegmented egg, only much more accentuated, viz. a dense, fine-grained perinuclear zone, and a less dense, more vacuolated peripheral zone, in which there is present a coarse, irregular network of deeply staining strands, recalling the framework of mitochondrial origin described by Van der Stricht ('04, '05) in the human ovum and that of Vesperugo. We have here in this differentiation of the cytoplasm, evidence of the occurrence of an intense metabolic activity which has resulted in a marked increase in the amount of deutoplasmic material present in the blastomeres as compared with that found in the ovarian egg or even in the unsegmented uterine egg. The blastomeres consequently present a somewhat dense opaque appearance when examined in the fresh state, their nuclei being partially obscured from view. Amongst the Eutheria, various observers (Sobotta, Van der Stricht, Lams and Doorme) have described a similar increase in the deutoplasmic contents of the egg after its passage into the Fallopian tube or uterus.

The second cleavage plane is also vertical and at right angles to the first. The resulting four equal-sized blastomeres viewed from the side (Pl. 2, fig. 19) are seen to be ovalish in outline, their lower ends being slightly narrower and more pointed than their upper ends, which diverge somewhat to enclose the lower part of the yolk-body. Seen from one of

the poles, in optical section (figs. 20, 21), they appear triangular with rounded corners and centrally directed apices. The space occupying the polar diameter, which they enclose is the cleavage cavity. The blastomeres are now somewhat less opaque than those of the 2-celled stage, so that their nuclei, excentrically situated nearer their upper ends and enclosed in the central granular zone of the cytoplasm, can now be fairly distinctly made out in the fresh egg.

The arrangement of the blastomeres at this stage is exceedingly characteristic, and is identical with that of the blastomeres in the corresponding stage of Amphioxus or the frog, but is quite different from that normal for the 4-celled stage of the Eutheria. They lie disposed radially or meridionally around the polar diameter, occupied by the cleavage cavity, their thicker upper ends partially surrounding the yolk-body. Selenka figures a precisely similar arrangement in his 4-celled stage of Didelphys, so that we may conclude it holds good for the Marsupials in general.

Whilst, then, in Marsupials the first two cleavage planes are vertical or meridional, and at right angles to each other, and the first four blastomeres are arranged radially around the polar diameter (radial type of cleavage), in the Eutheria such is never the case, at all events normally, so far as is known. In the Eutheria the first four blastomeres form, or tend to form, a definite cross-shaped group, as the result apparently of the independent division of the first two blastomeres in two different planes at right angles to each other, the division planes being meridional in the one, equatorial in the other.[1] This pronounced difference in the spatial relations of the first four blastomeres in the Metatheria and Eutheria is a feature of the very greatest interest and importance, since it is correlated with and in part conditions the marked dissimilarity which we meet with in the later developmental occurrences in the two groups, in particular in the mode of formation of the blastocyst in the two.

[1] Compare in this connection Assheton's remarks ('09, pp. 232–233), which have appeared since this chapter was written.

Moreover, so far as the Eutheria are concerned, it affords us, I believe, a striking and hitherto unrecognised example of a phenomenon to which Lillie ('99) has directed attention, viz. adaptation in cleavage.

Fig. 22 shows a horizontal section through the 4-celled stage, and fig. 23 a vertical section of the same. The blastomeres in their cytoplasmic characters essentially resemble those of the 2-celled stage, but the peripheral deutoplasmic network is here more strongly developed, and it is especially worthy of note that it is more marked towards the lower poles of the blastomeres (fig. 23), as also appears to be the case in the 2-celled stage. The shell-membrane measures in thickness ·0072 mm.

The next succeeding (third) cleavages are again meridional, each of the four blastomeres becoming subdivided vertically into two, not necessarily synchronously. Fig. 53. Pl. 6, shows a side view, and fig. 54 a view from the lower pole of a 6-celled egg, two of the blastomeres of the 4-celled stage having divided before the other two. The blastomeres have moved apart, and now form an open ring approximately equatorial in position, and surrounding the central cleavage space, the upper opening of which is occupied by the yolk-body. I have failed to obtain a perfectly normal 8-celled stage, nevertheless the evidence clearly shows that the first three cleavage generations in Dasyurus are meridional and equal, and that the resulting eight equal-sized blastomeres form an equatorial ring in contact with the inner surface of the sphere formed by the zona and shell-membrane.

Whilst, then, the first three cleavage generations are meridional and equal, the succeeding divisions (fourth cleavage generation), on the contrary, are equatorial and unequal, each of the eight blastomeres becoming divided into a smaller, more transparent upper cell, with relatively little deutoplasm, and a larger, more opaque lower cell with more abundant deutoplasmic contents. In this way there is formed an exceedingly characteristic 16-celled stage, consisting of two

superimposed rings, each of eight cells. The upper ring of smaller and clearer cells partially encloses the yolk body, and is situated entirely in the upper hemisphere of the sphere formed by the egg-envelopes. The lower ring of larger, more opaque cells lies approximately in the equatorial region of the said sphere. This 16-celled stage is figured in fig. 55, Pl. 6, as seen from the side, and in fig. 56 as seen from the upper pole, both figures being taken from a spirit egg ·37 mm. in diameter. The marked differences in the cells of the two rings are well brought out in the micro-photographs reproduced as figs. 24, 25, and 26, Pl. 2. Figs. 24 and 25 represent horizontal sections of an egg ·38 mm. in diameter, the former showing the eight cells of the lower ring, and the latter the eight cells of the upper ring. Fig. 26 shows a vertical section through an egg also of a diameter of ·38 mm., but with seventeen cells, one of the original eight cells of the upper ring having divided and one being in process of division. The section passes through the yolk-body (*y.b.*), which is seen as a faintly outlined structure lying in contact with the zona between the two cells of the upper ring (*f.c.*).

The shell-membrane in eggs of this 16-celled stage has attained a thickness of ·0075 mm., and the albumen layer has been almost completely absorbed, so that the zona now lies practically in apposition with the shell-membrane, the two together forming a firm resistant sphere, to the inner surface of which the blastomeres are closely applied. The separation between the zona and shell-membrane seen in the figures is largely, if not wholly, artificial.

The average measurements of the cells of the two rings in the ·38 mm. egg, figured in figs. 24 and 25, are as follows :

	Upper ring cells.	Lower ring cells.
Diameter .	·06 × ·058 mm. .	·09 × ·064 mm.
Vertical height	·095 mm. .	·115 mm.
Nucleus . .	·0165 mm. .	·02 mm.

These measurements demonstrate at a glance the distinct difference in size which exists between the cells of the two rings, whilst the cytoplasmic differences between them are

equally evident from an inspection of the micro-photographs, figs. 24–26. In the larger cells of the lower ring (fig. 24, *tr.ect.*) the nucleus (rich in chromatin and nucleolated) is surrounded by a perinuclear zone of clearer, coarsely vacuolar cytoplasm, outside of which is a densely granular deutoplasmic zone, which extends to within a short distance of the periphery of the cell-body. In the smaller cells of the upper ring (fig. 25, *f.c.*) the cytoplasm is coarsely reticular, with a tendency to compactness round the nucleus, and its contained deutoplasmic material is spare in amount as compared with that of the lower cells, being mainly located in a quite narrow peripheral zone. The upper cells thus appear relatively clear as compared with the dense, opaque-looking lower cells (fig. 26).

It becomes evident, then, that we have to do here, in this fourth cleavage generation, with an unequal qualitative division of the cytoplasm of the blastomeres of the 8-celled stage. Just such a division as this we should expect if the deutoplasmic material were mainly aggregated towards the lower poles of the dividing cells. The evidence shows that this is actually the case. In the 2-celled and especially in the 4-celled eggs we have already seen that the deutoplasmic network is already most strongly developed towards the lower poles of the blastomeres. This polar concentration of the deutoplasm reaches its maximum in blastomeres of the 8-celled stage, and confers on these an obvious polarity. Although I failed to obtain normal examples of the latter stage, I have fortunately been able to observe the characters of the blastomeres in sections of eggs with twelve, thirteen, and fourteen cells respectively.

In the 12-celled egg (Pl. 6, fig. 57), measuring ·38 mm. in diameter, four of the eight original blastomeres are still undivided; the remaining four have undergone division unequally and qualitatively, one but recently, so that $4 + (4 \times 2) = 12$. The undivided blastomeres are large (average diameter, ·11 × ·076 mm.) and ovoidal in form, their lower ends being thicker than their upper, and they exhibit a well-

marked polarity. The nucleus lies excentrically in the upper half of the cell, just above the equator, and is surrounded by a finely granular zone of cytoplasm, outside which is a thin irregular ring of deutoplasmic material. The cytoplasm of the apical part of the cell is clear and relatively free from deutoplasm; that of the lower half, on the other hand, is so rich in deutoplasm as to appear quite dense and opaque. The conclusion is therefore justified that the blastomeres of the 8-celled stage possess a definite polarity, which has been acquired as the result of the progressive concentration of deutoplasmic material at their vegetative poles during the cleavage process. Division, in the equatorial plane, of cells so constituted must necessarily be unequal and qualitative, so far at least as the cytoplasm is concerned.

In the 13-celled stage three of the original eight blastomeres are in process of division, and five have already divided unequally and qualitatively, so that $3 + (5 \times 2) = 13$, and in the 14-celled stage two of the original blastomeres are in division and six have already divided: $2 + (6 \times 2) = 14$.

The significance to be attached to this characteristic unequal and qualitative division of the blastomeres of the 8-celled stage to form two superimposed cell-rings, markedly differentiated from each other, we shall presently consider. Meantime I may categorically state the conclusions I have reached in regard thereto. The wall of the blastocyst in Dasyurus is at its first origin, and for some considerable time thereafter, unilaminar throughout its entire extent, and I regard the upper cell-ring of the 16-celled stage as giving origin to the formative or embryonal region of the unilaminar wall, the lower cell-ring as furnishing the extra-embryonal or non-formative remainder of the same. I shall therefore refer to the upper cell-ring and its derivatives as formative or embryonal, and to the lower cell-ring and its derivatives as non-formative or extra-embryonal.

The formative or embryonal region furnishes the embryonal ectoderm and the entire entoderm of the vesicle, and I accordingly conclude that it is the homologue of the embryonal knot

or inner cell-mass of the Eutherian blastocyst. The non-formative or extra-embryonal region directly gives origin to the outer extra-embryonal layer of the bilaminar blastocyst wall, i.e. to that layer which in the Sauropsida and Prototheria is ordinarily termed the extra-embryonal ectoderm. I regard it as such, and as the homologue of the so-called trophoblast (or as I prefer to term it, the "trophoblastic ectoderm" or "tropho-ectoderm") of the Eutherian blastocyst.

A word or two here before concluding this section by way of summary, as to the condition of the enclosing egg-envelopes. During the sojourn of the egg in the uterus the albumen is gradually resorbed, and by about the 16-cell stage it has all but completely disappeared, thus permitting the zona to come into direct apposition with the inner surface of the shell-membrane. The shell-membrane itself increases very considerably in thickness during cleavage, and by the 16-celled stage had practically reached its maximum, viz. ·0075–·008 mm., i.e. it is nearly five times thicker than that of the ovum which has just entered the uterus. The thickened shell-membrane by itself is firm and resistant, and it becomes still more so by the application of the zona to its inner surface, the two together forming a spherical supporting case round the segmenting egg, to the inner surface of which the blastomeres become closely applied.

The existence of such a firm supporting envelope round the Marsupial egg is, in my view, a feature of very great ontogenetic significance, and one which must be taken into account in any comparison of the early developmental occurrences in the Metatheria and Eutheria. As the sequel will show, the mode of formation of the blastocyst in these two sub-classes is fundamentally different, and in my opinion the explanation of this difference is to be found in the retention by the Metatheria of a relatively thick resistant shell-membrane, and its complete disappearance amongst the Eutheria.

2. Formation of the Blastocyst.

It is characteristic of the Marsupial that the cleavage-cells proceed directly to form the wall of the blastocyst, without the intervention of a morula stage, as in the Eutheria.

The fifth cleavages are meridional, each of the eight cells of the two rings of the 16-celled stage becoming subdivided vertically into two, so that there results a 32-celled stage consisting of two rings, each composed of sixteen cells. As might be expected, the smaller less yolk-rich cells of the upper ring tend to divide more rapidly than the larger yolk-laden cells of the lower ring, but the difference in the rate of division of the two is only slight. I have, for example, sections of a 17-celled stage (that already referred to, fig. 26) consisting of nine formative cells ($= 6 + [1 \times 2] + 1$ in division) and eight non-formative cells, and also of a 31-celled stage (Pl. 6, fig. 59, seen from lower pole; cf. also fig. 60, showing a side view of another 31-celled egg, both eggs ·375 mm. in diameter), consisting of sixteen formative and fifteen non-formative cells, of which one is in process of division. But I have also preparations of 32-celled eggs with an equal number of formative and non-formative cells, showing that the latter may make up their leeway, the former resting meantime. On the other hand, the cells of the two rings may divide more irregularly, as evidenced by a stage of about forty-two cells, consisting approximately of twenty-three formative cells ($= 9 + [7 \times 2]$) and nineteen non-formative ($= 13 + [3 \times 2]$). Whatever the rate of division, the important point is that the division planes are always radial to the surface, so that all the resulting blastomeres retain a superficial position in contact with the inner surface of the supporting sphere formed by the zona and shell-membrane. In apposition with the continuous surface afforded by that, the blastomeres, continuing to divide, gradually spread round towards the poles, the descendants of the upper or formative cell-ring gradually extending towards the upper pole marked by the yolk-body, whilst those of the

lower or non-formative cell-ring similarly spread towards the lower pole. As the blastomeres divide and spread they become smaller and more flattened, and gradually cohere together, and so in this way they eventually give origin to a complete unilaminar layer lining the inner surface of the sphere formed by the egg-envelopes. It is this unilaminar layer which constitutes the wall of the blastocyst.

The just completed blastocyst of Dasyurus is a spherical fluid-filled vesicle measuring about ·4 mm. in diameter (Pl. 3, figs. 27–29, Pl. 6, figs. 61, 62), and invested externally by the thin zona and the shell-membrane (·0075–·0078 mm. in thickness). The albumen layer has completely disappeared, and the shell-membrane, zona, and cellular wall are from without inwards in intimate apposition. The smallest complete vesicles which I have examined measure ·39 mm. in diameter (figs. 27, 61), and in one of these I find the cellular wall consists approximately of about 108 cells. In four other eggs of the same diameter and from the same female the wall of the blastocyst is as yet incomplete at the lower pole (fig. 31, *l.p.*), and in these, rough counts of the cells yielded the following respective numbers—89, 93, 121, 128. In another also incomplete blastocyst of the same batch, ·41 mm. in diameter (fig. 32), the cellular wall consists of about 130 cells. The largest complete blastocyst in this same batch measured ·49 mm. in diameter, so that we have a range of variation in size of the just completed blastocyst extending from ·39 to ·49 mm.

The unilaminar wall of the blastocyst consists of a continuous layer of more or less flattened polygonal cells (figs. 27–29, 61, 62) lying in intimate contact with the zona, itself closely applied to the shell-membrane. Over the lower hemisphere the non-formative cells are on the whole larger and plumper than the formative cells of the upper hemisphere, and in surface examination they appear somewhat denser owing to the fact that they possess much more marked perinuclear zones of dense cytoplasm than do the formative cells (cf. fig. 63, representing a ·6 mm. vesicle). In sections, however, this latter difference is much less obvious, indeed,

is hardly, if at all, detectable, so that one has to depend partly on the relative thickness of the cells, partly, and, indeed, mainly, on the yolk-body in determining which hemisphere is which.

The blastocyst cavity is tensely filled by a coagulable fluid derived from that poured into the uterine lumen through the secretory activity of the uterine glands. Also situated in the blastocyst cavity, in contact with the inner surface of the wall in the region of the upper pole, is the spherical yolk-body (fig. 29, *y.b.*). It becomes overgrown and enclosed in the blastocyst cavity as the result of the completion of the cellular wall over the upper polar region, much in the same sort of way as the yolk in the meroblastic egg becomes enclosed by the peripheral growth of the blastoderm. In the majority of my sections of early blastocysts the yolk-body has been dragged away from contact with the formative cells through the coagulation of the albuminous blastocystic fluid, and lies more or less remote from the wall enclosed by the coagulum, except on the side next the upper hemisphere (fig. 31, *y.b.*, *c.g.*). In two instances, one of which is shown in fig. 32, I find the yolk-body had become so firmly attached to one of the formative cells that the coagulum formed during fixation failed to detach it, and only succeeded in drawing it out to a pear-shape.

The yolk-body, it may here be mentioned, persists for a considerable time in the blastocyst cavity; I have found it shrunken indeed, but still recognisable, in relation to the embryonal area in vesicles 4·5–6 mm. in diameter. And there may even appear within it peripherally, irregular strands which stain deeply with iron-hæmatoxylin and which recall those forming the peripheral deutoplasmic network of the early blastomeres. Eventually, however, it seems to disappear, its substance passing into the blastocystic fluid, so that, as already remarked, it fulfils in this indirect way its original destiny.

Normally the cavity of the just completed blastocyst contains no cellular elements whatever. In one otherwise perfectly normal blastocyst (·39 mm. diam.) I find present,

however, a small spheroidal body ·028 mm. in diameter, composed of glassy-looking cytoplasm enclosing a central deeply staining granule. This I interpret as a cell or cell-fragment which has been accidentally separated off from the wall, and which has undergone degeneration. In later blastocysts such cellular bodies exhibiting more or less evident signs of degeneration are of fairly common occurrence. They are of no morphological significance.

Selenka's "Blastopore."—Normally the wall of the blastocyst is first completed over the upper hemisphere, in correspondence with the fact that the formative cells not only divide somewhat more rapidly than the non-formative but have a smaller extent of surface to cover, since the upper cell-ring from which they are derived lies about midway between the upper pole of the sphere formed by the egg-envelopes and the equator of the same, whilst the lower cell-ring from which the non-formative cells arise is approximately equatorial in position. We thus meet with stages in the formation of the blastocystic wall such as are represented in surface view on Pl. 3, fig. 30, and in section in figs. 31 and 32, in which the blastocystic cavity, prior to the completion of the cellular wall over the lower polar region, is more or less widely open below. There can be no doubt, I think, but that this opening corresponds to that observed by Selenka in his 42-celled "gastrula" of Didelphys and regarded by him as the blastopore, since he believed the entoderm arose from its lips. My observations conclusively show that it has no connection whatever with the entoderm, this layer arising from the formative region of the upper hemisphere, and that it is a mere temporary opening of no morphological significance, blastoporic or other. Prior to the completion of the wall at the upper pole a corresponding opening is temporarily present there also. Both owe their existence to the characteristic way in which the blastocyst wall is formed by the spreading of the products of division of the two cell-rings of the 16-celled stage towards opposite poles in contact with the surface provided by the enclosing egg-envelopes.

I have met with one specimen, an incomplete blastocyst ·39 mm. in diameter (belonging to the same batch as the other blastocysts referred to in this section[1]), in which the lower hemisphere would appear to have been completed before the upper, for the yolk-body lies in contact with the zona in the region where the cellular wall is as yet absent, and that the yolk-body has not been secondarily displaced is proved by a micro-photograph of the specimen in my possession (taken immediately after its transference to the fixing solution), in which the yolk-body is seen to lie at the unclosed pole in exactly the same position as in the sections.

In connection with this exceptional specimen, it may be recalled that Selenka, in his 68-celled "gastrula" of Didelphys (fig. 10, Taf. xvii), figures the wall as complete at the lower pole, the "blastopore" having already closed, but as still incomplete at the upper pole, there being present a small opening leading into the blastocyst cavity. In the 42-celled "gastrula" (fig. 8, Taf. xvii) this same opening and the "blastopore" as well are present. The occurrence of these openings at opposite poles, and the general agreement in the constitution of the blastocyst wall (larger, more yolk-rich cells at lower pole, smaller, less yolk-rich cells at upper), in the corresponding stages in Didelphys and Dasyurus justify the conclusion that the blastocyst of the former develops in the same way as does that of the latter. It is worthy of remark, however, that the just completed blastocyst of Didelphys appears to be considerably smaller than that of Dasyurus. Selenka unfortunately gives no measurements of his early stages, but I have calculated from the figure, the magnification of which is given, that the 68-celled blastocyst has a diameter of about ·137 mm. The corresponding stage of Dasyurus measures about ·39 mm., and is therefore nearly three times as large.

[1] This batch, from female 2 B. 16 . vii . '01, comprised altogether twenty-eight eggs, of which some eighteen were normal complete and incomplete blastocysts (·39–·49 mm. in diameter) and ten abnormal, four of these being unsegmented ova.

Selenka's Urentodermzelle.—Whilst the 42- and 68-celled blastocysts described by Selenka may be regarded as normal so far as the occurrence of polar openings and the constitution of their wall are concerned, I hold them to be abnormal in respect of the presence in each of a single large yolk-laden cell, regarded by Selenka as entodermal in significance. It is well to point out that Selenka was not able actually to determine the fate of this cell; he merely presumed that it took part in the formation of the definitive entoderm. No such cell occurs in normal blastocysts of Dasyurus at any stage of development, and in my opinion Selenka's "urentodermzelle" is none other than a retarded and displaced blastomere, i.e. a blastomere which has failed for some reason to divide, and which has become secondarily enclosed by the products of division of its fellows, and I am strengthened in this interpretation by the occurrence in an abnormal blastocyst of Dasyurus of just such a large cell as that observed by Selenka. The vesicle in question is one of the batch already referred to, and measured ·397 mm. in diameter. The cellular wall (fig. 37) is apparently normal, but is incomplete at one spot, and the gap so left is occupied by a large binucleated cell, rich in deutoplasm and measuring ·12 × ·072 mm. (fig. 37, *abn.*). This cell corresponds in its size and cytoplasmic characters with a non-formative blastomere of about the 16-celled stage, and I regard it simply as a blastomere which has failed to undergo normal division. In another abnormal blastocyst (·39 mm. diam.) from the same batch, the cellular wall appears complete and normal, but the blastocyst cavity contains a group of about sixteen spherical cells averaging about ·032 mm. in diameter, and in yet another abnormal egg of the same diameter and batch there is present an incomplete layer of flattened cells over one hemisphere, and towards the opposite pole of the egg-sphere there occurs a group of spherical cells of variable size and some of them multinucleate. In this abnormal egg it appears as if the formative cells had divided in fairly normal fashion, whilst the non-formative cells had failed to do so.

Chapter IV.—Growth of the Blastocyst and Differentiation of the Embryonal Ectoderm and the Entoderm.

1. Growth of the Blastocyst.

In the preceding chapter we have seen that the cleavage process in Dasyurus results in the formation of a small spherical vesicle, about ·4 mm. in diameter, which consists, internally to the investment formed by the apposed zona and shell-membrane, simply of a cellular wall, unilaminar throughout its entire extent, and enclosing a fluid-filled cavity normally devoid of any cellular elements. The stage of the just completed blastocyst is followed by a period of active growth of the same, and it is a noteworthy feature in the development of Dasyurus that during this time the blastocyst undergoes no essential structural change, but remains unilaminar until it has reached a diameter of from 4·5 to 5·5 mm. Even during cleavage, the egg of Dasyurus increases in diameter, partly owing to the thickening of the shell membrane, partly, and, indeed, mainly, as the result of the accumulation of uterine fluid under pressure within the egg-envelopes, but the increase due to these causes combined is relatively insignificant, being only about ·1 mm. As soon, however, as the cellular wall of the blastocyst is completed, rapid growth sets in, under the influence of the hydrostatic pressure of the fluid, which tensely fills the blastocyst cavity, with the result that the small relatively thick-walled blastocyst becomes converted into a large extremely thin-walled vesicle, but beyond becoming very attenuated, the cellular wall during this period of active growth undergoes no essential change, and retains its unilaminar character until the blastocyst, as already mentioned, has reached a diameter of from 4·5 to 5·5 mm. In vesicles of about this size there become differentiated from the formative cells of the upper hemisphere the embryonal ectoderm and the entoderm, and this latter layer then gradually spreads round inside the non-formative (extra-embryonal ectodermal) layer of the lower hemisphere so as to

form a complete lining to the blastocyst, which thereby becomes bilaminar. Such a marked enlargement of the blastocyst prior to the differentiation of the embryonal ectoderm and entoderm as is here described for Dasyurus does not apparently occur, so far as known, in other Marsupials : in Perameles, for example, the embryonal ectoderm and the entoderm are in process of differentiation in vesicles a little over 1 mm. in diameter (v. p. 77), in Macropus these two layers are already fully established in a vesicle only ·8 mm. in diameter (v. p. 79), and much the same holds good for Trichosurus and Petrogale. It is paralleled by the marked growth which in the Monotremes follows the completion of the blastocyst and which precedes the appearance of embryonal differentiation. It must be remembered, however, that the growing blastocyst in the Monotreme is bilaminar and not unilaminar as in Dasyurus, owing to the fact that the entoderm is established as a complete layer at a very much earlier period than is the case in the latter. I am nevertheless inclined to regard the attainment by the Dasyurus blastocyst of a large size, prior to the differentiation of the embryonal ectoderm and the entoderm, as a more primitive condition than that found in other Marsupials. The pronounced hypertrophy which the uteri of Dasyurus undergo during the early stages of gestation, an hypertrophy which appears to be proportionately greater than that met with in other forms,[1] is no doubt to be correlated with the presence in them of such a considerable number of actively growing blastocysts.

Selenka states (Heft 5, p. 180) that he examined seven blastocysts of Dasyurus "$\frac{3}{4}$ mm." in diameter, taken from a female fifteen days after copulation. He describes their structure as follows: "Man unterscheidet (1) eine sehr zarte äussere, homogene Haut (Granulosamembran), (2)

[1] For example, the uteri of a female (5, 18 . vii . '01) from which I obtained twenty-one normal vesicles, 4·5–6 mm. in diameter, with the embryonal area definitely established, measured as follows : Left uterus, 4·5 × 4·7 × 1·4 cm. (fourteen vesicles) ; right uterus, 4·5 × 4·2 × 1·45 cm. (seven vesicles and one shrivelled).

darunter ein Lager von Ektodermzellen, welche im Gebiete des Embryonalschildes prismatich, am gegenüberliegenden Pole nahezu kubisch, im übrigen abgeplattet erscheinen, (3) ein inneres zusammenhängendes Lager von abgeflachten Entodermzellen." This description, apart from the reference to the thin shell-membrane, is entirely inapplicable to blastocysts of Dasyurus of the mentioned size which I have studied.

I have examined a practically complete series of vesicles of Dasyurus ranging from ·4 mm. to 4 mm. in diameter and all of them without exception are unilaminar.

Of vesicles under 1 mm. diameter I possess serial sections of more than two dozen, ranging from ·5 mm. to ·8 mm. in diameter, and obtained from three different females. These differ structurally in no essential respect from the just completed blastocysts. A surface view of a blastocyst ·6 mm. in diameter is shown in fig. 63, Pl. 6; in this the difference in the cytoplasmic characters of the cells of opposite hemispheres is clearly brought out, the non-formative cells of the lower hemisphere having much more marked perinuclear zones of dense cytoplasm (deutoplasm) than the formative cells of the upper hemisphere; moreover, the former cells tend to be of larger superficial extent than the latter. Fig. 34, Pl. 3, represents a section of a blastocyst ·57 mm. in diameter, and fig. 35 a section of one ·73 mm. in diameter. These blastocysts differ in no essential way from the ·43 mm. blastocyst represented in fig. 33. As in the latter, the cellular wall is unilaminar throughout, but both it and the shell-membrane have undergone considerable attenuation. Moreover in these blastocysts, apart from the clue afforded by the shrivelled yolk-body, it is practically impossible to determine from the sections which is morphologically the upper hemisphere and which the lower. In fig. 36, from a ·6 mm. blastocyst, on the other hand, the cells of the hemisphere opposite the yolk-body (*y.b.*) are larger than those of the hemisphere adjacent to which that body is situated. In the ·57 mm. blastocyst the shell-membrane has a thickness of ·0052 mm., in the ·73 mm. blastocyst it measures ·0045 mm., and in a ·84 mm. blastocyst

·0026 mm. The zona is now no longer recognisable as an independent membrane. In blastocysts of this stage of growth a variable number of small spherical cells or cell-fragments are frequently met with in the blastocyst cavity, usually lying in contact with the inner aspect of the cellular wall (fig. 34, *i.c.*). In some blastocysts such structures are absent, in others one or two may be present, in yet others numbers of them may occur. They may be definitely nucleated, but this is exceptional; more usually they contain one or more deeply staining granules (of chromatin?), or are devoid of such. They are of no morphological importance, and I think there can be no doubt that they represent cells or fragments of cells which have been separated off from the cellular wall during the process of active growth. They are of common occurrence in later blastocysts, and it is possible the so-called "yolk-balls" observed by Selenka in Didelphys are of the same nature.

If we pass now to vesicles from 1 to 3 or 3·5 mm. in diameter, we find the wall still unilaminar, but considerably more attenuated than it is in the blastocysts last referred to. In a vesicle with a diameter of 1·24 mm. the shell-membrane has a thickness of about ·0015 mm., whilst the cellular wall has a thickness of only ·0045 mm. In a 3·5 mm. vesicle the shell-membrane measures about ·0012 mm., whilst the cellular wall ranges from ·0018 to ·0048 mm. in thickness. A small portion of the wall of a vesicle, 2·4 mm. in diameter, is shown in Pl. 6, fig. 64. In these later vesicles I have failed to detect, either in surface examination of the vesicles in toto or in sections, any regional differences between the cells indicative of a differentiation of the wall into upper or formative, and lower or non-formative, hemispheres. Everywhere the wall is composed of flattened, extremely attenuated cells, polygonal in surface view, and all apparently of the same character. It might therefore be supposed that the polarity, which is recognisable in early blastocysts, and which is dependent on the pronounced differences existent between the cells of the upper and lower rings of the 16-celled stage, is of no funda-

mental importance, since it apparently becomes lost at an early period during the growth of the blastocyst. Such an assumption, however, would be very wide of the mark, as I hope to demonstrate in the next section of this paper, and, indeed, in view of the facts already set forth, is an altogether improbable one.

Reappearance of Polar Differentiation in the Blastocyst Wall.—Following on the period of what may be termed the preliminary growth of the blastocyst, in the course of which the original polar differentiation in the blastocyst wall apparently becomes obliterated, is an extremely interesting one, during which that differentiation again becomes manifest. In view of the fact (1) that the fourth cleavage in Dasyurus is of the nature of a qualitative cytoplasmic division, and (2) that approximately one half or rather less of the unilaminar vesicle wall is formed from the eight smaller and less yolk-rich cells of the upper ring of the 16-celled stage, and its remainder from the eight larger more yolk-rich cells of the lower ring, it thus becomes a question of the first importance to determine if we can the significance of that differentiation.

Amongst the Eutheria, it has been conclusively shown by various observers (Van Beneden, Heape, Hubrecht, Assheton, and others) that there occurs during cleavage an early separation of the blastomeres into two more or less distinctly differentiated groups, one of which eventually, by a process of overgrowth, completely encloses the other. The peripheral cell-group or layer forms the outer extra-embryonal layer of the wall of the later blastocyst (the trophoblast of Hubrecht, or trophoblastic ectoderm as I prefer to term it). It therefore takes no direct part in the formation of the embryo, and may be distinguished as non-formative. The enclosed cell-group, termed the inner cell-mass or embryonal knot, gives rise, on the other hand, to the embryonal ectoderm as well as to the entire entoderm of the vesicle, and may accordingly be distinguished as formative. May it not be, then, that we have here at the fourth cleavage in Dasyurus a separation of the

blastomeres into two determinate cell-groups, respectively formative and non-formative in significance, entirely comparable with, and, indeed, even more distinct than that which occurs during cleavage in the Eutheria? I venture to think that the evidence brought forward in this paper conclusively justifies an answer in the affirmative to that question.

If we assume that the upper cell-ring of the 16-celled stage in Dasyurus is formative in destiny and the lower cell-ring non-formative, then we might naturally expect to find in the unilaminar wall of the later blastocyst some differentiation indicative of its origin from two distinct cell-groups, and indicative at the same time of the future embryonal and extra-embryonal regions. Now just such a differentiation does, as a matter of fact, become evident in vesicles 3·5 to 4·5 mm. in diameter. We have already seen that the wall in early blastocysts ·4 to ·8 mm. in diameter exhibits a well-marked polar differentiation in correspondence with its mode of origin from the differentiated cell-rings of the 16-celled stage, its upper hemisphere or thereabouts consisting of smaller cells, poor in deutoplasm, its remainder of larger cells, rich in deutoplasm. In later blastocysts, 1–3 mm. or more in diameter, it is no longer possible to recognise this distinction—at all events I have failed to observe it—but if we pass to blastocysts 4·5 mm. in diameter, in which the wall is still unilaminar, we find on careful examination of the entire vesicle under a low power that there is now present a definite continuous line, which encircles the vesicle in the equatorial region so as to divide its wall into two hemispherical areas (Pl. 4, fig. 38, *j.l.*). If we remove and stain a portion of the wall of such a vesicle, including this line, and examine it microscopically (figs. 42–46), it becomes apparent at once, from the disposition of the cells on either side of the line, that we have to do with a sutural line or line of junction produced by the meeting of two sets of cells, which are pursuing their own independent courses of growth and division. The cells never cross the demarcation line from the one side to the other, but remain strictly confined

to their own territory, so that we are justified in regarding the vesicle wall as composed of two independently growing zones. Now the existence of two such independent zones in the unilaminar wall is, to my mind, only intelligible on the view that they are the products of two originally distinct, predetermined cell-groups, and if this be admitted, then I think we are justified in concluding, in view of the facts already set forth, that the two zones in question are derived, the one from the upper cell-ring of the 16-celled stage, the other from the lower ring; that, in other words, they represent respectively the upper and lower hemispheres of the early blastocysts.

If, now, we find that the embryonal ectoderm and the entoderm arise from one of these two regions of the unilaminar wall, whilst the other directly forms the outer extra-embryonal layer of the later (bilaminar) vesicle, then we must designate the former region as the upper or formative, and the latter as the lower or non-formative. Further, bearing in mind the characters of the cells of the two rings of the 16-celled stage, I think we are justified in holding that the formative region is derived from the ring of smaller, less yolk-rich cells, and the non-formative region from the ring of larger, more yolk-rich cells, even if it is impossible to demonstrate an actual genetic continuity between the constituent cells of these two rings and those forming the independently growing areas of the later blastocyst. I have recently re-examined a series of vesicles, measuring 1·5–1·8 mm. in diameter, obtained from a female killed in 1906, and I have so far found it impossible, either in the entire vesicle or in portions of the wall stained and mounted on the flat, to distinguish between the cells over opposite hemispheres. Thus the only actual guide we have for the determination of the poles in such vesicles is the yolk-body, and though the latter is liable to displacement, it is worthy of record that I have several times found it in relation to the formative area in vesicles 4·5–6 mm. in diameter, but never in relation to the non-formative region. This evidence is, therefore, so far as it goes, confirmatory of

the conclusion reached above, viz. that the formative hemisphere is derived from the smaller-celled ring of the 16-celled stage. On that conclusion is based my interpretation of the poles in the unsegmented ovum, and of the two cell-rings of the 16-celled stage as respectively upper and lower.

Of vesicles over 1 mm. in diameter, the smallest in which I have been able to detect the sutural line above referred to measure 3·25 mm. in diameter. In three lots of vesicles, 3·5 mm. in diameter from three different females, I have failed to recognise it, whilst in two other lots, respectively 3·75 mm. (average) and 4 mm. in diameter, the line appears to be in course of differentiation as in the 3·25 mm. vesicles. A portion of the wall of one of the 3·5 mm. vesicles just referred to is shown in Pl. 4, fig. 41, and a portion of the wall of the 3·25 mm. stage, including the sutural line, in fig. 42. Both vesicles were fixed in the same fluid, viz. picro-nitro-osmic acid. Comparison of the two figures reveals the existence, quite apart from the presence of the junctional line in fig. 42, and its absence in fig. 41, of certain more or less obvious differences between them. In fig. 41 the cells are larger, and their cytoplasmic bodies are inconspicuous, being fairly homogeneous and lightly staining. In fig. 42, on the contrary, the cell-bodies are strongly marked, the cytoplasm being distinguishable into a lighter-staining peripheral zone, and a much more deeply staining perinuclear zone, showing evidence of intense metabolic activity. This latter zone is more or less vacuolated, and contains, besides larger lightly staining granules, numerous smaller ones of varying size, stained brown by the osmic acid of the fixative. In the 4 mm. vesicles the cells show precisely the same characters; in the 3·75 mm. vesicles, which were fixed in a picro-corrosive-acetic fluid, the granules are absent from the cytoplasm, otherwise the cells are similar to those of the other two. Mitotic figures are common. The sutural line is recognisable in all three sets of vesicles (3·25, 3·75, and 4 mm.) (fig. 42, *j.l.*), but I cannot be certain that it runs continuously round, and it appears to have a rather more sinuous course than in later blastocysts. The cells of the two regions

of the blastocyst wall, separated by the sutural line, differ somewhat in their characters. On one side of the line (fig. 42, *tr.ect.*) the cells appear to be on the whole slightly larger, and of more uniform size than they are on the other, and they also stain somewhat more deeply. Comparison with later blastocysts shows that the region of more uniform cells is non-formative, that of less uniform, formative. At this stage, however, the differences between the cells of the two regions are as yet so little pronounced that it is practically impossible in the absence of the sutural line to say to which hemisphere an isolated piece of the wall should be referred.

I am inclined to regard the sutural line in these vesicles as being in course of differentiation, and judging from the disposition of the cells on either side of it, I think its appearance is to be correlated with the marked increase in the mitotic activity of the cells of the two hemispheres which sets in in vesicles of 3–4 mm. diameter. The preliminary increase in size of the blastocyst up to about the 3 mm. stage might be described as of a passive character, i.e. it does not appear to be effected as the result of the very active division of the wall-cells, but is characterised rather by a minimum of mitotic division and a maximum of increase in surface extent of the cells, due to excessive stretching consequent on the rapid imbibition of uterine fluid. Once, however, the requisite size has been attained, the cells of the unilaminar wall commence to divide actively, and doubtless as the outcome of that wave of activity, the sutural line makes its appearance between the two groups of independently growing cells.

On the inner surface of the blastocyst wall, especially in the region of the formative hemisphere, there are present in these vesicles numbers of small deeply staining cells of spherical form, and containing osmicated granules similar to those in the wall-cells. They may occur singly or in groups, and appear to me to be of the same nature as the internal cells of the earlier blastocyst. In addition to these cells, there are present clusters of cytoplasmic spheres, staining similarly to the spherical cells, and apparently of the nature of fragmenta-

tion products formed either directly from the wall-cells or from these internal cells.

2. Differentiation of the Embryonal Ectoderm and the Entoderm.

After the preliminary growth in size of the blastocyst is completed, the next most important step in the progressive development of the latter is that just dealt with, involving the appearance of the sutural line, with resulting re-establishment of polar differentiation in the blastocyst wall. Following on that, we have the extremely important period during which the embryonal ectoderm and the entoderm become definitely established.

For the investigation of the earlier phases of this critical period I have had at my disposal a large number of unilaminar blastocysts derived from three females, distinguished in my notebooks as β, 25 . vii . '01, with fifteen vesicles of a maximum diameter of 4·5 mm.; 8 . vii . '99, with twelve vesicles, 4·5 mm. in diameter; and 6 . vii . '04, with twenty-two vesicles, 4·5 and 5 mm. in diameter. These three lots of vesicles may for descriptive purposes be designated as '01, '99, and '04 respectively.

The '01 vesicles are distinctly less advanced than the other two. The sutural line is now, at all events, definitely continuous, and can readily be made out in the intact vesicle with the aid of a low-power lens (Pl. 4, fig. 38, *j.l.*), but the differences between the cellular constituents of the two hemispheres which it separates are much less obvious than they are in the '99 and '04 vesicles. Here, again, one hemisphere forming half or perhaps rather more of the entire vesicle is distinguished from the other by the greater uniformity and the slightly deeper staining character of its constituent cells (figs. 43 and 44, *tr. ect.*). This hemisphere, subsequent stages show, is the lower or non-formative hemisphere. It is characterised especially by the striking uniformity in the size of its cells. Over the opposite hemisphere, the upper or formative one (figs. 43 and 44, *f.a.*), the

cells are more variable in size, the nuclei thus appearing less uniformly and less closely arranged, and they stain, on the whole, somewhat less deeply than those of the lower hemisphere. The non-formative cells are on the average smaller than the largest of the formative cells, but they are more uniform in size, and their nuclei thus lie at more regular distances apart, and appear more closely packed. They are also richer in deutoplasmic material, and so stain rather more deeply than the formative cells. Sections show that the cellular wall is unilaminar throughout its extent, and that, whilst it is somewhat thicker than that of 3·5 mm. vesicles, it is still very attenuated, its thickness, including the shell-membrane, ranging from ·004 to ·008 mm. I have examined a number of series of sections taken through portions of the wall known to include the sutural line, and find it quite impossible to locate the position of the latter; indeed, I cannot certainly distinguish between the formative and non-formative regions.

In the blastocyst cavity, lying in contact with the inner surface of the wall, and most abundant in the region of the formative hemisphere, there are present numbers of deeply staining spherical cells with relatively small nuclei similar to those described in connection with the 3·25 mm. vesicles. They occur singly or in groups, and may appear quite normal or may show more or less evident signs of degeneration. Their nuclei may stain deeply and homogeneously, or may be represented by one or two deeply staining granules, vacuoles may occur in their cytoplasm, and spherical cytoplasmic masses of very variable size, with or without deeply staining granules of chromatin, may occur along with them. In sections and preparations of the wall of these and other 4·5 mm. vesicles there are to be found, in both the formative and non-formative hemispheres, small localised areas from which such spherical cells are being proliferated off in numbers together. Pl. 5, fig. 47, from the formative hemisphere of an '04 vesicle shows one of the most marked examples of such proliferative activity that I have encountered. A similar but smaller proliferative

area occurs on the non-formative hemisphere of the same vesicle.

These spherical cells are, I am convinced, of no morphological importance, and are destined sooner or later to degenerate. They have certainly nothing to do with the entoderm, the parent-cells of that layer arising exclusively from the formative hemisphere and not from cells such as these, which are budded off from both hemispheres. The fact that they are, in unilaminar vesicles, more numerous over the formative hemisphere may perhaps be taken as an indication of the greater mitotic activity of the formative as compared with the non-formative cells.

The Primitive Entodermal Cells.—Following closely on the stage represented by these '01 blastocysts is the extremely important one constituted by the '99 and '04 vesicles before referred to. This stage is the crucial one in primary germ-layer formation, and marks the transition from the unilaminar to the bilaminar condition, since in it the entodermal cells are not only distinctly recognisable as constituents of the formative region, but are to be seen both in actual process of separation from the latter and as definitely internal cells, frequently provided with, and even connected together by, pseudopodial-like processes of their cell-bodies. Such cells are already present in the '01 vesicles (fig. 71), and probably also in the blastocysts in which the sutural line first makes its appearance, but are much less conspicuous than in these older blastocysts.

The '99 blastocysts are distinctly more advanced than the '01 batch and are just a little earlier than the '04 lot. The former measured, as already mentioned, 4·5 mm. in diameter, the latter 4·5 and 5 mm. (the majority being of the latter size). In my notes on the intact '99 vesicles I find it stated that one hemisphere, forming rather less than half of the entire extent of the vesicle wall, appeared somewhat denser than the other, the sutural line marking the division between the two. I naturally inferred at the time that the denser hemisphere corresponded to the embryonal region of the

Eutherian blastocyst and the less dense to the extra-embryonal region of the same, but just the reverse proves to hold true for the '04 vesicles, the formative hemisphere in these appearing less dense than the non-formative. I cannot now test my former inference by direct observation since I do not appear to have any of the '99 vesicles left intact, but amongst my in toto preparations of the vesicle wall I find one labelled as from the "lower pole" which unmistakably belongs to the formative hemisphere, hence I conclude that the denser and slightly smaller region which I originally regarded as formative is really non-formative, a conclusion which brings the '99 vesicles into agreement with the '04 batch.

In these latter vesicles the sutural line and the two regions of the wall can be quite readily made out on careful examination under a low power with transmitted light. The one region appears slightly denser (darker) and has more closely arranged nuclei (i. e. is composed of smaller cells) than the other. On the average this denser region appears to be rather the less extensive of the two; the two regions may be about equal; on the other hand the denser may be the smaller. Examination of stained preparations of the wall demonstrates that the darker hemisphere is non-formative, the lighter, formative. It would therefore seem that in certain of these '04 vesicles the formative region has grown more rapidly than the non-formative.

In stained preparations of the wall both of the '99 and '04 vesicles, the differences between the two hemispheres are now so well marked that there is no difficulty in referring even an isolated fragment to its proper region. The non-formative hemisphere differs in no essential way from that of the '01 vesicles, and as in these, is readily distinguishable from the formative by the much greater uniformity in the size and staining properties of its cells (fig. 45), as well as by the fact that there are no primitive entodermal cells such as occur in relation to the formative hemisphere, in connection with it. Its constituent cells are on the average distinctly smaller than

the largest of the formative ; their nuclei lie nearer each other, with the result that in surface examination of the blastocyst the non-formative region appears rather denser than the formative. In in toto preparations of the wall the former usually stains darker than the latter (fig. 45), but this is not always the case ; in fig. 46, from an '04 vesicle, there is practically no difference in this respect between the two regions ; in yet others of my preparations of '99 vesicles the formative region has stained more deeply than the non-formative.

The formative hemisphere in the earlier blastocysts of this particular developmental stage was described (ante, p. 51) as differing from the non-formative in that its constituent cells were much less uniform in character than those of the latter. This same feature, but in much enhanced degree, characterises the formative region of the vesicles under consideration, for it can now be definitely stated that the latter region is constituted by cells of two distinct varieties, viz. (1) more lightly staining cells which form the chief constituent of the formative region, its basis so to speak, and which are on the average larger than those of the other variety, and (2), a less numerous series of cells, distinctly smaller than the largest cells of the former variety, and with denser, more granular and more deeply staining cytoplasm, and frequently met with in mitotic division (cf. Pl. 6, fig. 65). The two varieties of cells are intermingled promiscuously, the smaller cells occurring singly and in groups but in a quite irregular fashion, so that here and there we meet with patches of the wall composed exclusively of the larger cells.

The evidence presently to be adduced shows that the larger cells furnish the embryonal ectoderm, and that the smaller cells give origin to the primitive entodermal cells from which the definitive entoderm arises. The smaller cells may therefore be regarded as entodermal mother-cells. Whether these latter cells are progressively formed from the larger cells simply by division, or whether the two varieties become definitely differentiated from each other at a particular stage in

development, must for the present be left an open question. Of the actual existence in the unilaminar formative region of these '99 and '04 blastocysts of two varieties of cells, respectively ectodermal and entodermal in significance, there can be no doubt. In preparations of the formative region, however, whilst one can without hesitation identify certain cells as being in all probability of ectodermal significance and others as prospectively entodermal (cf. figs. 65, 66), it must be admitted that one is often in doubt as to whether one is dealing with small ectodermal cells or with genuine entodermal mother-cells. It is, therefore, hardly to be wondered at that I have not yet been able to satisfactorily determine at what precise period the entodermal mother-cells first become differentiated, though judging from the facts that in the earliest vesicles in which the sutural line is recognisable one region of the wall already differs from the other in the less uniform size of its constituent cells, and that internally situated entodermal cells are already present in small numbers in the '01 vesicles (fig. 71), I incline to the belief that it will probably be found to about coincide with the first appearance of the sutural line. To this question I may perhaps be able to return at some future time.

In addition to the presence of these entodermal mother-cells, which enter directly into its constitution, the formative region of the '99 and '04 blastocysts is characterised by the occurrence on its inner surface of definitely internal cells, which generally agree with the former cells as regards size and staining properties and are evidently related to them. It is these internally situated cells which directly give origin to the definitive entoderm of the later blastocysts, and one need, therefore, have no hesitation in applying to them the designation of primitive entodermal cells. They are exclusively found in relation to the formative hemisphere, and appear in *in toto* preparations as flattened, darkly staining cells closely applied to the inner surface of the unilaminar wall, and disposed quite irregularly, singly, and in groups. They vary greatly in number in blastocysts of even the same batch, but on the

whole are most abundant in the '04 series, and they also exhibit a remarkable range of variation in shape. They may have a perfectly distinct oval or rounded outline (figs. 67, 71, 72), or, as is more frequently the case, they may lack a determinate form and appear quite like amœboid cells owing to their possession of cytoplasmic processes of markedly pseudopodial-like character (fig. 69). Frequently, indeed, the cells are connected together by the anastomosing of these processes, so that we have formed in this way the beginnings at least, of a cellular reticulum (figs. 68, 69, 70).

The question now arises, How do these primitive entodermal cells originate from the small, darkly staining cells of the unilaminar formative region designated in the foregoing as the entodermal mother-cells? I can find no evidence that the primitive entodermal cells are formed by the division of the mother-cells in planes tangential to the surface; on the contrary, all the evidence shows that we have to do here with an actual inward migration of the mother-cells, with or without previous mitotic division, such inward migration being the outcome of the assumption by the mother-cells, or their division products, of amœboid properties; in other words, the evidence shows that the formation of the entoderm is effected here not by simple delamination (using that term in the sense in which it was originally employed by Lankester), but by a process involving the inward migration, with or without previous division, of certain cells (entodermal mother-cells) of the unilaminar parent layer, a process comparable with that found in certain Invertebrates (Hydroids) and distinguished by Metschnikoff as "gemischte Delamination."

In this connection it has to be remembered that the cells of the unilaminar wall of the blastocyst are under considerable hydrostatic pressure, and, in correlation therewith, tend to be tangentially flattened, though the flattening in this stage is much less than in the earlier blastocysts. From a series of measurements made from an '04 vesicle, I find that over the formative region the ratio of the breadth to the thickness of the cells varies from 6 : 1 to 2 : 1, and even to 3 : 2. On the

whole cells of the type indicated by the ratio 6 : 1 predominate, and we should hardly expect to find such cells dividing tangentially. In fact, the only undoubted examples of such division I have met with occur in the single abnormal vesicle present in the '04 batch. In this particular vesicle, which had a diameter of 3 mm. and was thus smaller than the others, there was present on what appeared to correspond to the formative hemisphere of the normal blastocyst a well-defined and conspicuous ovalish patch, 1·23 × ·99 mm. in diameter.[1] Sections show that over this area the cells of the unilaminar wall are much enlarged and more or less cubical in form, their thickness varying from ·012 to ·019 mm. These cubical cells exhibit distinct evidence of tangential division, both past and in progress. But in normal vesicles, whilst mitotic figures are quite commonly met with in the cells of the formative region (in which, indeed, they are more numerous than in those of the non-formative region), I have failed to find in my sections after long-continued searching even a single spindle disposed directly at right angles to the shell-membrane; the mitotic spindles lie disposed either tangentially to the surface or obliquely thereto.

For the determination of the mode of origin of the primitive entodermal cells, it is absolutely necessary to study both *in toto* preparations of the formative region, i.e. small portions of the unilaminar wall stained and mounted on the flat, and sections of the same. Sections alone are, on the whole, distinctly disappointing so far as the question under discussion is concerned, and, indeed, give one an altogether inadequate idea of the primitive entodermal cells themselves, seeing that practically all one can make out is that

[1] Curiously enough, amongst the '99 vesicles there also occurred a single small one, likewise 3 mm. in diameter, and with a thickened patch 1·28 × 1 mm. in diameter, quite similar in its character to that described in the text. I am as yet uncertain whether the thickened area in these two vesicles represents the whole of the formative hemisphere of normal blastocysts or only a hypertrophied part of the same, or whether, indeed, it may not represent the retarded non-formative hemisphere.

there are present, in close apposition with the inner surface of the unilaminar wall, small, darkly staining cells, apparently quite isolated from each other and usually of flattened form (figs. 73, 74, 76, *ent.*). One has only to glance at a well-stained in toto preparation of the formative region (cf. fig. 70) to realise how inadequate such a description of the primitive entoderm cells really is.

Sections nevertheless do yield valuable information on certain points. Besides affording the negative evidence of the absence of tangential divisions and the positive evidence that the primitive entodermal cells are actually internal (figs. 73, 74, 76), they show that growth of the wall in thickness has already set in, and that it is most marked over the formative region, though the thickness attained by the cells is as yet very unequal (figs. 73–76). Measurements taken from an '04 vesicle show that over the non-formative region (fig. 77) the cells vary in thickness from ·006 to ·009 mm., whilst over the formative region the range of variation is greater, viz. from ·006 to ·013 mm., so that we may conclude that the latter region is on the average thicker than the former (cf. figs. 73–76, with fig. 77 depicting a small portion of the non-formative region). It is still impossible to determine the position of the sutural line, even in sections of fragments of the wall known to contain it.

The entodermal mother-cells are not very readily recognisable in sections. In fig. 75, however, which is drawn from an accurately transverse section through the formative region of an '04 vesicle, there is depicted what is undoubtedly an entodermal mother-cell (*ent.*). The interesting point about this particular cell is that its cell-body, whilst still intercalated between the adjoining cells of the unilaminar wall, has extended inwards so as to directly underlie one of the wall-cells. Division of such a cell as this would necessarily result in the production of an internally situated cell with all the relations of one of the primitive entodermal type. The inwardly projecting spheroidal cell situated immediately to the left (in the figure) of the one just referred to, I also

regard as an entodermal mother-cell. Cells of this type are not infrequently met with in sections; they usually stain somewhat deeply, and are often found in mitosis.

The evidence obtainable from the study of in toto preparations conclusively proves that some at all events of the primitive entodermal cells are actually derived from the entodermal mother-cells much in the way suggested above, whilst others of the primitive entodermal cells are directly formed from mother-cells which bodily migrate inwards.

Fig. 65, Pl. 6, represents a small portion of the formative region of an '04 vesicle viewed from the inner surface. In the centre of the figure, surrounded by the larger, lighter staining (ectodermal) cells of the wall, is a smaller cell in the telophases of division, the cytoplasm of which is granular and stains deeply. That cell unmistakably forms a constituent of the unilaminar wall. I regard it as an entodermal mother-cell. Fig. 66 shows another cell of the same character in the anaphases of division, which likewise forms a constituent of the unilaminar wall, but which differs from the corresponding cell in fig. 65 in that its cytoplasmic body has extended out on one side (lower in the figure), so as to directly underlie part of an adjacent ectodermal cell. In other words we have here a surface view of the condition represented in section in fig. 75, only the entodermal mother-cell depicted therein is not actually in process of division. Fig. 67, taken from the same preparation as fig. 65, shows what I take to be the end result of the division of such a cell as is represented in the two preceding figures. Here we see two small deeply staining cells towards the centre of the figure, which from their disposition and agreement in size and cytological characters are manifestly sister-cells, and the products of division of just such an entodermal mother-cell as is represented in fig. 65, or, better, fig. 66. The one cell (upper in the figure) is more angular in form and manifestly still lies in the unilaminar wall; the other (lower in the figure) is ovalish in form and is no longer a constituent of the unilaminar wall, but is on the contrary a free cell, definitely internal both to the

latter and to its sister-cell. It is, in fact, a primitive entodermal cell, as comparison with fig. 68 proves, and that it has been formed by the division of a mother-cell situated in the unilaminar wall can hardly, I think, be doubted. Its sister-cell, which is still a constituent of the wall, would presumably have migrated inwards some time later.

It is to be noted that the primitive entodermal cell referred to above and those depicted in figs. 71 and 72 are definitely contoured, ovalish and rounded cells, entirely devoid of processes. In these respects they differ markedly from the entodermal cells shown in figs. 68–70, which are very variable in form owing to their possession of more or less elongated pseudopodial-like processes. It might therefore be inferred that the formation of these processes only takes place after the entodermal cells have become definitely internal. Such an inference, however, would be incorrect, for I have abundant evidence showing that such processes may be given off from the entodermal mother-cells whilst they are still constituents of the wall. In *in toto* preparations, it is often difficult to determine with certainty whether a particular entodermal cell still enters into the constitution of the unilaminar wall or not. In the portion of the formative region of a '04 vesicle depicted in fig. 70, however, I am satisfied that all the entodermal cells therein shown (they are readily distinguishable by their smaller size and more deeply staining character) are, with the possible exception of the one on the extreme right, at least partially intercalated between the larger ectodermal cells of the wall. Some of them are entirely situated in the wall; others have extended inwards in varying degree so as to partially underlie the ectodermal cells. It is these latter entodermal cells in particular which exhibit the cytoplasmic processes above referred to. As the figure shows, these processes have all the characters of pseudopodia; they vary in size, form, and number from cell to cell, individual processes may be reticulate and their finer prolongations may anastomose with those of others, and they are formed of cytoplasm, less dense and rather less deeply staining than that of the

cell-bodies from which they arise. Attention may be specially directed to the cell towards the left of the figure (marked *ent.*). Here we have an entodermal cell whose cytoplasmic body is evidently still partially intercalated between the cells of the wall, but which is, at the same time, prolonged inwards (towards the left) so as to underlie the adjoining ectodermal cell. From this inward prolongation there are given off two slender processes, one short and tapering, the other very much longer; this latter, after becoming very attenuated, gradually widens to form an irregular fan-shaped expansion, sucker-like in appearance, and produced into several slender threads, which is situated adjacent to the nucleus of the ectodermal cell on the extreme left. Then from the right side of the same cell there is given off a small inwardly projecting bulbous lobe which may well be the start of just such another process as arises from the left side. Processes of the peculiar sucker-like type just described, formed of a slender elongated stem and a distal expanded extremity from which delicate filamentous prolongations are given off, are abundantly met with in preparations, and strikingly recall the pseudopodia of various Rhizopoda. They are seen in connection with other entodermal cells in fig. 70, and with many of those in fig. 68. I regard them as veritable pseudopodia. Towards the right side of fig. 70 the two entodermal cells there situated stand in direct protoplasmic continuity by means of two slender connecting threads, whilst the upper of these two cells is again joined by a very fine process to the irregular pseudopodial expansion which arises from one of the two entodermal cells situated nearer the middle of the figure, and that same expansion is directly connected with the second of the two entodermal cells just mentioned, so that we have here established the beginning of a cell-network, prior to the complete emancipation of its constituent entodermal elements from the unilaminar wall. We have, then, clear evidence that the entodermal elements in Dasyurus, prior to their separation from the unilaminar formative region are capable of exhibiting amœboid activity, since not only may

they send lobose prolongations of their cytoplasmic bodies inwards below the adjacent ectodermal cells, but they may emit more or less elongated processes of indubitable pseudopodial character, which similarly lie in contact with the inner surface of the wall-cells. Furthermore, we have evidence that these pseudopodial processes may anastomose with each other so as to initiate the formation of an entodermal reticulum, whilst the cells from which they arise are still constituents of the unilaminar wall—an especially noteworthy phenomenon. Certain of the primitive entodermal cells, as we have seen, are at first devoid of such processes, but since they all eventually form part of a continuous reticulum, it is evident that the entodermal elements are capable of emitting pseudopodial processes as well after as before their separation from the formative region.

Finally, in view of the fact that the entodermal mother-cells depicted in fig. 70 are not actually in process of division, and therein differ from those of figs. 65 and 66, we may conclude that the formation of the primitive entodermal cells is effected either with or without the previous division of the mother-cells.

If we admit, as I think on the evidence we must admit, that the entodermal cells in Dasyurus are endowed with amœboid properties, then we are relieved of any further difficulty in regard to the mechanism of their inward migration from the unilaminar wall. Doubtless, in the case of those entodermal mother-cells which do not undergo division, the precocious formation of the above-described pseudopodial processes which spread out from the cells like so many suckers considerably facilitates their direct detachment from amongst the cells of the wall. In the case of those primitive entodermal cells which originate as the direct products of division of the mother-cells, it no doubt depends on a variety of circumstances (e.g. actual form of the dividing cell, direction of the spindle, etc.) whether they exhibit amœboid activity precociously (i.e. before their actual separation), or only at a later period.

The entoderm varies considerably in its degree of diffe-

rentiation in different vesicles of this stage, and even in different parts of the formative region of one and the same vesicle. In some vesicles there are relatively few primitive entodermal cells, in others they are much more abundant. Fig. 68, from the formative region of an '04 vesicle, shows a typical patch of them and illustrates very well the highest stage of differentiation which they attain in these vesicles. The entodermal cells therein depicted all appear to be definitely internal, and it is especially worthy of note that the portion of the unilaminar wall in relation to them is composed exclusively of the larger, lighter staining cells. It is these cells which directly form the embryonal ectoderm of the blastocysts next to be described. The entodermal cells are obviously amœboid in character (observe especially the cells near the middle of the figure), and are in active process of linking themselves together into a cellular reticulum. In fig. 69 is shown a small portion of the formative region of another '04 vesicle. A single entodermal mother-cell in process of division occurs in position in the unilaminar wall, which is otherwise composed of ectodermal cells, whilst internally there are present three entodermal cells, already linked together by their pseudopodial processes. The two lowermost cells afford especially striking examples of amœboid activity, the elongated pseudopodial process of the cell on the left terminating in a well-marked reticulation in definite continuity with the corresponding, but shorter and thicker process of the cell on the right.

3. Establishment of the Definitive Embryonal Area.

Following directly on the stage represented by the '04 blastocysts described in the preceding section is one designated in my list as 5, 18 . vii . 01 and referred to here as 5, '01. It comprises twenty-two blastocysts obtained from a female killed fifteen days after coition and all normal, with the exception of one which was shrivelled, and all in precisely

the same stage of development. They measured from 4·5 to 6 mm. in diameter.

In this stage the formative region of the preceding blastocysts has become transformed into the definitive embryonal area (embryonic shield, Hubrecht) as the result of the completion of that process of inward migration of the entodermal mother-cells which we saw in progress in the vesicles last described, and the consequent establishment of the entoderm as a continuous cell-layer underlying and independent of the embryonal ectoderm constituted by the larger passive cells of the original unilaminar formative layer.

In the entire blastocyst (Pl. 4, fig. 39) the embryonal area is quite obvious to the naked eye as the more opaque, hemispherical region, forming rather less than half the entire extent of the vesicle wall; the larger remainder of the same is formed by the much more transparent, non-formative or extra-embryonal region. Sections of the entire blastocyst show (1) that the embryonal area is bilaminar over its entire extent, its outer layer consisting of embryonal ectoderm, already somewhat thickened, its much thinner inner layer consisting of entoderm, partly still in the form of a cellular reticulum, and (2) that the extra-embryonal region is still unilaminar throughout and composed of a relatively thin layer of flattened cells (extra-embryonal or trophoblastic ectoderm, trophoblast [Hubrecht])[1] (Pl. 8, fig. 78). The entoderm is co-extensive at this stage with the embryonal ectoderm, and terminates in a wavy, irregularly thickened, free edge (Pl. 5, fig. 49), which over most of its extent either directly underlies or extends very slightly beyond the line of junction between the embryonal and extra-embryonal ectoderm. The junctional line is thus not very easily seen. In fig. 48, however,

[1] In consonance with my conviction that this layer is homologous both with the so-called trophoblast of Eutheria and the extra-embryonal ectoderm of Prototheria, and in view of the theoretical signification which Hubrecht now insists should be attached to the term "trophoblast," and which I am wholly unable to accept, I venture to suggest as an alternative name for this layer that of "tropho-ectoderm."

a small portion of the line shows with sufficient distinctness, I think, to demonstrate its identity with that of the preceding stage.

The vesicle wall in all my sections of this stage appears to be somewhat thinner than that of the '04 blastocysts, but apart from this apparently variational difference the present blastocysts are almost exactly intermediate between the latter and those next to be described.

The embryonal ectoderm (fig. 78, *emb. ect.*) appears in section fairly uniformly thickened, though its cells are still of the flattened type. In surface view in in toto preparations (cf. fig. 48), they exhibit the same polygonal form and lightly staining qualities as the larger cells of the formative region of the '04 blastocysts, which we have already identified as prospective embryonal ectodermal cells. The junctional line between the embryonal ectoderm and the extra-embryonal is now for the first time readily distinguishable in sections (fig. 78). The extra-embryonal ectoderm (tropho-ectoderm) (Pl. 5, figs. 48 and 49, Pl. 8, fig. 78, *tr. ect.*) differs in no essential respect from the corresponding layer in the '04 blastocysts.

The entoderm in these blastocysts is exceedingly closely adherent to the inner surface of the embryonal ectoderm and cannot be removed therefrom by artificial means. It varies slightly in its character in different vesicles and in different parts of its extent in the same vesicle. Mostly it appears as a continuous thin cell-layer (figs. 49 and 78, *ent.*), but here and there patches occur in which the cells form a reticulum quite similar to that shown in fig. 68 of the preceding stage.

The next stage (designated in my list as 8 . vi . 01), and the last of Dasyurus that need be described in the present communication, comprises eleven vesicles (5–5·5 mm. in diameter), in which the embryonal area is conspicuous and distinctly in advance of that of the preceding vesicles, but is still devoid of any trace of embryonal differentiation (Pl. 4, fig. 40; Pl. 8, fig. 79).

The embryonal area is hemispherical in form (its greatest

diameter varying from 3·5 to 4 mm.) in all except two of the blastocysts, in which it is elongate, with longer and shorter diameters. It occupies about a third or less of the entire extent of the vesicle wall, and thus appears relatively smaller than that of the preceding (5, '01) vesicles. The entoderm now extends for a distance of about 1 mm. beyond the limits of the area, so that in the entire vesicle (fig. 40) three zones differing in opacity are distinguishable, viz. the dense hemispherical zone at the upper pole, constituted by the embryonal area; below that, a less dense, narrow annular zone, formed of extra-embryonal ectoderm and the underlying peripheral extension of the entoderm; and finally, the still less dense hemispherical area, forming the lower hemisphere of the blastocyst and constituted solely by extra-embryonal ectoderm. Thus approximately the upper half of the blastocyst is bilaminar, the lower half unilaminar. Sections show that the embryonal ectoderm (fig. 79, *emb. ect.*) is now a quite thick layer of approximately cubical cells, whilst the extra-embryonal ectoderm (*tr. ect.*) is formed of relatively thin flattened cells. The line of junction between the two is perfectly obvious, both in sections (fig. 79) and in surface view (Pl. 5, fig. 50). The embryonal ectodermal cells, though much thicker than the extra-embryonal, are of less superficial extent; their nuclei therefore lie closer together than those of the latter, moreover they are larger, stain more deeply, and are more frequently found in division, all of which facts testify to the much greater growth-activity of the embryonal as compared with the extra-embryonal ectoderm at this stage of development (cf. fig. 50, *emb. ect.* and *tr. ect.*; in the preparation from which this micro-photograph was made the entoderm underlying the embryonal ectoderm has been removed, whilst it is still partially present over the extra-embryonal ectoderm).

The entoderm (fig. 79, *ent.*) over the region of the embryonal area is readily separable as a quite thin membrane, and is then seen to consist of squamous cells, polygonal in outline, and either in direct apposition by their edges or connected together by minute cytoplasmic processes. Beyond the

embryonal area, however, its peripheral extension below the extra-embryonal ectoderm is much less easily separable in the intact condition (cf. fig. 50), because of its greater delicacy due to the fact that it has here largely the form of a cellular reticulum. In this extra-embryonal region the entodermal cells are frequently found in mitosis. It would appear, then, that the entoderm is first laid down in the region of the embryonal area as a cellular reticulum, which later becomes transformed into a continuous cell-membrane, and that its peripheral extension over the inner surface of the extra-embryonal ectoderm is the result of the growth and activity of its own constituent cells.

This peripheral growth continues until there is formed eventually a complete entodermal lining to the blastocyst cavity. The rate of growth appears to be somewhat variable. In a series of primitive streak vesicles (6–6·75 mm. in diameter) the lower third of the wall is, I find, still unilaminar. In another series of vesicles of the same developmental stage (4·5–6 mm. in diameter) a unilaminar area is present at the lower pole, varying from 1 × ·5 mm. in diameter to as much as 4 mm. Even in vesicles 7–7·5 mm. in diameter a unilaminar patch may still occur at the lower pole, but in vesicles 8·5 mm. in diameter (stage of flat embryo) the entodermal lining appears always to be complete.

The Origin of the Entoderm in Eutheria.—The remarkable facts relative to the origin of the entoderm in Dasyurus which I have been able to place on record in the preceding pages, thanks to the large size attained by the blastocyst prior to the differentiation of the formative germ-layers and to the circumstance that the formative cells are not arranged, as they are in Eutheria, in the form of a more or less compact cell-mass, but constitute a thin unilaminar cell-layer of relatively great extent which can easily be cut up with scissors, and which, after staining and mounting on the flat can be examined under the highest powers, throw, it seems to me, a new and unexpected light on the mammalian entoderm, and at the same time help to fill the considerable

gap which has hitherto existed in our knowledge of its early ontogenesis. Although the mode of origin of the entoderm in Dasyurus would appear, in the present state of our knowledge, to find its closest parallel, not amongst vertebrates, but in certain invertebrates (cf. the mode of origin of the entodermal cells from the wall of the blastula in Hydra as described by Brauer[1]), the observations of Assheton ('94) on the early history of the entoderm in the rabbit, when viewed in the light of the foregoing, seem to me to afford ground for the belief that phenomena comparable with those here recorded for Dasyurus will eventually be recognised as occurring also in Eutheria.

Hubrecht ('08), in his recent treatise on early Mammalian ontogeny, deals very briefly with the question of the origin of the entoderm in the latter group, merely stating that "from the inner cell-mass arises by delamination a separate lower layer which we designate as the entoderm of the embryo. These entoderm cells wander in radial direction along the inner surface of the trophoblast, which in many cases is thus soon transformed into a didermic structure. . . . When the entoderm has separated off by delamination from the embryonic knob, the remaining cells of the latter form the 'embryonic ectoderm,' which is thus situated between the entoderm and the trophoblast."

Assheton, in the paper just referred to, has given a careful account of the first appearance of the entodermal cells in the rabbit, and of what he believes to be the mode of their peripheral extension below the trophoblastic wall of the blastocyst. He shows that the inner cell-mass, at first spherical, gradually, as the blastocyst enlarges, flattens out below the "covering layer" of the trophoblast until it forms an approximately circular plate "nowhere more than two cells thick." During the process of flattening, cells are seen to jut out from the periphery of the mass; these eventually separate, and appear as rounded cells scattered irregularly over the inner surface of the trophoblast and "extending

[1] 'Zeitschr. f. wiss. Zool.,' Bd. lii, 1891.

over an arc of about 60° from the upper pole in all directions." These "straggling" cells, as Assheton terms them, as well as the innermost cells of the now flattened inner cell-mass, are regarded as hypoblastic and the outermost cells of the same as epiblastic (embryonic epiblast). "The hypoblast, as a perfectly definite layer, is formed by the time the blastodermic vesicle measures ·5 mm. in diameter, that is, about the 102nd hour after coition. It is not, however, as yet by any means a continuous membrane; it is a network or fenestrated membrane. For this reason, in section it appears to be represented by isolated cells lying beneath the embryonic disc (v. fig. 29, *Hy.*)" (cf. Dasyurus). In considering the question how the peripherally situated ("straggling") entodermal cells, which are undoubtedly derived from the inner cell-mass, "apparently wander round the inside of the blastodermic vesicle," he reaches the conclusion that this is not the result of amœboid activity or growth "in the sense of migration" on the part of these cells, but "is only an apparent growth round produced by the more rapid growth of a zone of the [trophoblastic] wall of the vesicle immediately surrounding the embryonic disc, in which zone the marginal cells of the inner mass lie." He is unable to find any evidence of the production of pseudopodial processes by these peripheral entodermal cells, the majority of them appearing at first to be quite isolated from each other and approximately spherical. "Certain of the cells here and there are connected by threads of protoplasm, but this, I think, is not a sign of pseudopodic activity, but merely indicates the final stage in division between the two cells." By the sixth day the hypoblast of the embryonic disc has assumed the form of a continuous membrane, composed of completely flattened cells, whilst the peripheral hypoblast cells have become more numerous, and "many of them, possibly all of them, are now undoubtedly connected by more or less fine protoplasmic threads." Such, in brief, is Assheton's account of the early history of the entoderm in the rabbit; it presents obvious points of agreement with my

own for Dasyurus, and I venture to think the agreement is even greater than would appear from Assheton's conclusions. In adopting the view that the more active growth of the region of the blastocyst wall immediately surrounding the inner cell-mass is the sole causal agent in effecting the separation and peripheral spreading of the entodermal cells, I cannot but feel, in view of his own description and figures and of my own results, that he has attributed a much too exclusive importance to that phenomenon and a much too passive rôle to the entodermal cells themselves. In Dasyurus the inward migration and the later peripheral spreading of the entodermal cells is effected without any such marked unequal growth of the blastocyst wall as occurs, according to Assheton, in the rabbit, as the direct outcome of their own inherent activity, and I believe the possession of a like activity characterises the entodermal cells of the rabbit. The evidence of Assheton's own fig. 40, which shows in surface view a portion of the vesicle wall with the peripheral entodermal cells in relation thereto, and which should be compared with my figs. 68 and 69, conclusively demonstrates, to my mind, the possession by these cells of amœboid properties, and thus support is afforded for the belief that the separation of the entodermal cells from the formative cell group (inner cell-mass) is here also the expression of an actual migration. Whether or not the strands of protoplasm which Assheton ('08, '09) describes as present in the sheep, pig, ferret, and goat, connecting the inner lining of the inner mass to the wall of the blastocyst, and which he interprets as tending "to show that the inner lining of the inner mass is of common origin with the wall of the blastocyst," are of any significance in the present connection, I cannot certainly determine.

4. Summary.

The results and conclusions set forth in the preceding pages of this chapter may be summarised as follows:

(1) The unilaminar wall of the blastocyst of Dasyurus con-

sists of two regions distinct in origin and in destiny, viz. an upper or formative region, derived from the upper cell-ring of the 16-celled stage, and destined to furnish the embryonal ectoderm and the entoderm and a lower or non-formative region derived from the lower cell-ring of the mentioned stage, and destined to form directly the extra-embryonal or trophoblastic ectoderm (tropho-ectoderm) of the bilaminar vesicle.

(2) The formative region, unlike the non-formative, is constituted by cells of two varieties, viz.: (i) a more numerous series of larger, lighter-staining cells destined to form the embryonal ectoderm, and (ii) a less numerous series of smaller, more granular, and more deeply staining cells, destined to give origin to the entoderm and hence distinguishable as the entodermal mother-cells.

(3) The entodermal mother-cells, either without or subsequently to division, bodily migrate inwards from amongst the larger cells of the unilaminar wall and so come to lie in contact with the inner surface of the latter. They thus give origin to the primitive entodermal cells from which the definitive entoderm arises. The larger passive cells, which alone form the unilaminar wall after the inward migration of the entodermal cells is completed, constitute the embryonal ectoderm.

(4) The entodermal cells as well before as after their migration from the unilaminar wall are capable of exhibiting amœboid activity and of emitting pseudopodial processes, by the anastomosing of which there is eventually formed a cellular entodermal reticulum underlying, and at first co-extensive with, the embryonal ectoderm.

(5) The definitive entoderm thus owes its character as a connected cell-layer primarily to the formation of secondary anastomoses between the pseudopodial processes emitted by the primitive entodermal cells (or entodermal mother-cells).

(6) The assumption by the entodermal cells of amœboid properties whilst they are still constituents of the unilaminar

wall affords an intelligible explanation of the mechanism of their inward migration.

(7) The entoderm is first laid down below the formative or embryonal region of the blastocyst; thence it extends gradually by its own growth round the inner surface of the unilaminar non-formative region so as to form eventually a complete entodermal lining to the blastocyst cavity. In this way the blastocyst wall becomes bilaminar throughout.

(8) The bilaminar blastocyst consists of two regions, respectively embryonal and extra-embryonal. The embryonal region (embryonal area) is constituted by an outer layer of embryonal ectoderm and the underlying portion of the entoderm, and the extra-embryonal, of the extra-embryonal or trophoblastic ectoderm (tropho-ectoderm), which is separated from the embryonal by a well-marked junctional line, together with the underlying portion of the entoderm, which is perfectly continuous with that below the embryonal ectoderm.

(9) The formative or embryonal region of the blastocyst in Dasyurus is from the first freely exposed, and at no time during the developmental period dealt with in this paper does there exist any cellular layer externally to it, i. e. a covering layer of trophoblast (Deckschicht, Rauber's layer) is absent and there is no entypy of the primary germ-layers (cf. p. 111).

Chapter V.—Some Early Stages of Perameles and Macropus.

The early material of Perameles and Macropus at my disposal comprises only a small number of stages, but is of special importance, since it enables me to demonstrate that so far as these particular stages are concerned, the early developmental phenomena in these forms are essentially the same as in Dasyurus, and thus affords ground for the belief that there is one common type of early development throughout the series of the Marsupialia. Moreover, it is of interest since it reveals the existence of what might be termed

specific differences in the early development of these Marsupials, especially in regard to the time of appearance of the entoderm. In Dasyurus, it will be remembered, the primitive entoderm cells first become definitely recognisable as internally situated cells in vesicles 4·5 mm. in diameter. In Perameles they occur in vesicles just over 1 mm. in diameter, while in Macropus they are already present in a blastocyst only ·35 mm. in diameter, so that it would appear that the entoderm is differentiated much earlier in the higher, more specialised types than in the more generalised forms. This difference in time of appearance of the entoderm is perhaps to be correlated with a difference in size of the ovarian ova in the three genera mentioned.

1. Perameles.

The earliest material of Perameles I possess consists of two eggs of P. obesula, which I owe to the skill and enthusiasm of my friend Mr. S. J. M. Morean, of Sydney. Egg A measures ·23 mm. in diameter, and egg B, ·24 × ·23 mm. The former consists of thirty-two cells, the latter of thirty. In both the shell-membrane has partially collapsed, but the general plan of arrangement of the blastomeres can still fairly readily be made out. Fig. 51, Pl. 3, represents a micro-photograph of a section of egg B, the better of the two. It shows the shell-membrane (nearly ·005 mm. thick) externally, considerable remains of the albumen between that and the deeply stained zona, and then, closely applied to the inner surface of the latter, the blastomeres arranged in the form of an inverted ∩, so as to enclose a central space, open below as the figure stands. This latter opening extends through the series, and it seems probable that there was a corresponding one opposite to it in the intact egg. Evidently we have here a stage in the formation of the blastocyst, in which the blastomeres are in course of spreading towards one or both of the poles of the sphere formed by the egg-envelopes,

just as happens in the corresponding stage of Dasyurus (cf. fig. 51 with fig. 31, though the latter represents a somewhat older stage in Dasyurus). The blastocyst-wall here appears relatively more extensive than in the 32-celled stage of Dasyurus, an apparent difference which may perhaps be accounted for by the difference in size of the respective eggs (·24 mm. as compared with ·36 mm.). The blastomeres situated adjacent to the opening and those on the right side of the figure tend to be more flattened and of greater superficial extent than the remainder, but I can recognise no difference in the cytological characters of the cells. The space or cleavage cavity enclosed by the blastomeres is partly occupied by a granular coagulum, and towards the opening there is present a lightly staining reticular mass, which recalls the yolk-body of Dasyurus, though I am not prepared to affirm that it is of that significance. The fixation of the specimen is not quite perfect.

My next stage of Perameles is constituted by a blastocyst of P. nasuta, for which I am again indebted to Mr. Moreau measuring in the preserved condition ·29 × ·26 mm. Fig. 52, Pl. 3, shows a section of this blastocyst. Structurally, it corresponds in all essential respects with the ·43 mm. blastocyst of Dasyurus, figured on the same plate (fig. 33). The blastocyst wall is complete and unilaminar throughout. It is distinguishable into two regions, a more extensive region over which the cells are large and flattened and a less extensive, composed of smaller but thicker cells (left side of fig. 52). In the early blastocysts of Dasyurus, it may be recalled, the evidence showed that the region of more flattened cells is formative in significance, that of more bulky cells, non-formative. It is possible the same holds good for this Perameles blastocyst. On the other hand, the structural condition of the stage next to be described rather supports the view that the smaller region, composed of plumper cells, is in this case formative. That view seems to me the more probable of the two, but there is a considerable difference in size between the present blastocyst and those next available, so that it is

impossible to decide this point with certainty. The blastocyst cavity is partly occupied by coagulum. There are no cells present in it, but the question of the presence of a yolk-body must remain open. The shell-membrane (·0045 mm. in thickness) and zona are in close apposition.

Following this early blastocyst, I have three vesicles of P. nasuta, two of them measuring 1·3 mm. in diameter, the other 1·1 mm. In their stage of development they agree pretty closely with the 4·5–5 mm. vesicles of Dasyurus, referred to in the preceding pages under the designation 6, '04, the entoderm being in process of differentiation. The formative region was readily distinguishable in the intact vesicles as a darker patch occupying about three eighths of the surface extent of the wall. In section (Pl. 8, figs. 80, 81) it is characterised by its greater thickness as compared with the non-formative or trophoblastic region, and by the presence below it of numbers of primitive entodermal cells. Compared with the corresponding stage in Dasyurus, the chief difference consists in the relatively much greater thickness of the cells of the formative region in the Perameles vesicle. The latter cells are here already more or less definitely cubical in shape, their thickness varying from ·09 mm. to ·015 mm., and altogether they form a layer of a much more uniformly thickened character than that of the 6, '04 vesicles of Dasyurus. The trophoblastic ectoderm (figs. 80, 81, *tr. ect.*) is composed of somewhat flattened cells, varying in thickness from ·005 to ·008 mm.

The primitive entodermal cells (figs. 80, 81, *ent.*) are present below the formative region in fair abundance, more especially around the periphery of the same, which may thus appear somewhat thickened (fig. 81). The cells vary in size from ·01 × ·007 mm. to ·024 × ·009 mm., and they stain on the whole somewhat more deeply than the formative cells, to whose under-surface they are closely applied. They occur singly and in groups. Mitotic figures are frequently met with in the cells of the formative area (observe the obliquely disposed figure in one of the formative cells in fig. 81), and

they also occur in the primitive entodermal cells. Examination of the sections leaves no doubt in one's mind as to the source of the entodermal cells. They are undoubtedly derived from the formative region of the vesicle wall. The shell-membrane has a thickness of about ·0027 mm.

2. Macropus.

Of Macropus the earliest stage I have examined is a blastocyst of M. ruficollis, ·25 × ·21 mm. in diameter. It is not in a quite perfect state of preservation, but is in a sufficiently good condition to enable me to say that the wall is complete and unilaminar throughout, just as in the ·29 × ·26 mm. blastocyst of Perameles. The shell-membrane has a thickness of about ·005 mm., and there are still remains of the albumen between it and the zona.

My next stage (figs. 82–85) is a blastocyst of the same species, ·35 mm. in diameter. It unfortunately suffered in preparation, but practically the whole of the formative area of the blastocyst wall and part of the trophoblastic ectoderm are comprised in the sections (Pl. 9, fig. 82), so that it is still possible to make out its chief structural features. In its stage of development this blastocyst closely agrees with the last described blastocysts of Perameles. The formative area of the wall is perfectly distinct in the sections because of its greater thickness and the presence below it of the primitive entodermal cells. It attains its greatest thickness (·027 mm.) peripherally, whilst it is thinnest centrally (·006 mm.), so that, taken as a whole, it is not quite such a uniformly thickened layer as is that of the Perameles blastocysts. Primitive entodermal cells are present below it, but not in great abundance (figs. 82, 84, 85, *ent.*). In fig. 83, a formative cell is seen in division, the axis of the spindle being oblique to the surface. The trophoblastic ectoderm (figs. 82, 83, *tr. ect.*) is composed of the usual flattened cells, and varies in thickness from ·005 to ·0067 mm.

In the blastocyst cavity, adjacent to the trophoblastic

ectoderm on the left side of fig. 82, there is visible a small spherical cell similar to the degenerate cells met with in blastocysts of Dasyurus.

My last stage of M. ruficollis comprises an excellently preserved blastocyst, measuring ·8 mm. in diameter, in which the embryonal ectoderm and the entoderm are definitely established. It thus corresponds to the 8, '01 stage of Dasyurus (blastocysts 5–5·5 mm. diameter). The embryonal area is circular and measures ·468 mm. in diameter. Its constituent cells are cubical and from ·008 to ·013 mm. in thickness, whilst the trophoblastic ectoderm is formed of flattened cells, ·006 mm. in thickness. The entoderm is present as a continuous layer of attenuated cells below the embryonal ectoderm, and it probably also forms a continuous layer below the trophoblastic ectoderm. Entodermal cells are certainly present over the lower polar region of the vesicle, but it is difficult to be certain from the sections whether or not they form a perfectly continuous layer. The shell membrane has a thickness of ·0026 mm.

I have a corresponding blastocyst of Petrogale penicillata ·915 mm. in diameter, with an oval, embryonal area ·525 × ·45 mm. in diameter, and a later blastocyst of M. ruficollis 1·46 mm. in diameter, with a circular embryonal area ·57 mm. in diameter.

Chapter VI.—General Summary and Conclusions.

The observations recorded in the preceding pages and the conclusions deducible therefrom may be summarised as follows:

(A) Ovum.—The uterine ovum of Dasyurus is characterised (1) by its large size relatively to those of Eutheria; (2) by the presence externally to the zona of a layer of albumen and a shell-membrane, both laid down in the Fallopian tube and homologous with the corresponding structures in the Monotreme ovum, the shell-membrane, like the shell of the latter, increasing in thickness in the uterus; (3) by its marked

polarity, its lower two thirds consisting of formative cytoplasm, dense and finely granular in appearance, owing to the presence of fairly uniformly distributed deutoplasmic material, and containing the two pronuclei, its upper third being relatively clear and transparent, consisting as it does of a delicate reticulum of non-formative cytoplasm, the meshes of which are occupied by a clear deutoplasmic fluid. Study of the process of vitellogenesis in ovarian ova demonstrates that this fluid represents surplus deutoplasmic material which has not been utilised in the upbuilding of the formative region of the ovum.

The fate of the clear non-formative portion of the ovum is a very remarkable one. Prior to the completion of the first cleavage, it is separated off from the formative remainder of the ovum as a spherical mass or yolk-body, which takes no direct part in development, though it becomes enclosed in the blastocyst cavity on completion of the blastocyst wall at the upper pole. Its contained deutoplasmic fluid is to be regarded as the product of an abortive attempt at the formation of a solid yolk-mass, such as is found in the Monotreme ovum. By its elimination the potentially yolk-laden telolecithal ovum becomes converted into a secondarily homolecithal, holoblastic one. All the evidence is held to support the conclusion that the Marsupials are descended from oviparous ancestors with meroblastic ova.

(B) Cleavage.—Cleavage begins in the uterus, is total, and at first equal and of the radial type. The first two cleavage planes are meridional and at right angles to each other. The resulting four equal-sized blastomeres lie disposed radially around the polar diameter like those of the Monotreme (not in pairs at right angles to each other as in Eutheria), and enclose a segmentation cavity open above and below, their upper ends partially surrounding the yolk-body. The third cleavage planes are again meridional, each of the four blastomeres becoming subdivided equally into two. The resulting eight cells form an equatorial ring in contact with the inner surface of the sphere formed by the egg-envelopes. They

contain deutoplasmic material, which is, however, located mainly in their lower halves. The ensuing fourth cleavages are equatorial, and in correlation with the just-mentioned disposition of the deutoplasm, are unequal and qualitative, each of the eight blastomeres becoming subdivided into an upper smaller and clearer cell, with relatively little deutoplasm fairly uniformly dispersed through the cytoplasm, and a lower larger, more opaque cell with much deutoplasm, mainly located in a broad zone in the outer portion of the cell-body. A 16-celled stage is thus produced in which the blastomeres are characteristically arranged in two superimposed rings, each of eight cells, an upper of smaller, clearer cells next the yolk-body, and a lower of larger, denser cells. The former is destined to give origin to the formative or embryonal region of the blastocyst wall, the latter to the non-formative or extra-embryonal region of the same.

(c) Formation of the Blastocyst.—There is in the Marsupial no morula stage as in Eutheria, the blastomeres proceeding directly to form the wall of the blastocyst. The cells of the two rings of the 16-celled stage divide at first meridionally and then also equatorially, the division planes being always vertical to the surface. The daughter-blastomeres so produced, continuing to divide in the same fashion, gradually spread towards opposite poles in contact with the inner surface of the firm sphere formed by the zona and the thickened shell-membrane. Eventually they form a complete cellular lining to the said sphere and it is this which constitutes the wall of the blastocyst. The latter is accordingly unilaminar at its first origin, and it remains so in Dasyurus until it has attained, as the result of active growth accompanied by the imbibition of fluid from the uterus, a diameter of 4–5 mm. It consists of two parts or regions, distinct in origin and in destiny, and clearly marked off from each other in later blastocysts by a definite junctional line approximately equatorial in position, viz. an upper, embryonal or formative region derived from the upper cell-ring of the 16-celled stage, and a lower, extra-embryonal or non-

formative region derived from the lower cell-ring of the same stage.

(D) Later History of the Two Regions of the Blastocyst Wall (for details see pp. 72–74).—From the embryonal region are derived the embryonal ectoderm and the entire entoderm of the vesicle. I conclude, therefore, that it is the homologue of the inner cell-mass or embryonal knot of the Eutherian blastocyst. The extra-embryonal region directly furnishes the outer extra-embryonal layer of the vesicle wall, i.e. the outer layer of the omphalopleure and chorion of later stages. Assuming, as the facts of comparative anatomy and palæontology entirely justify us in doing, that the Mammals are monophyletic and of reptilian origin, and further assuming that the fœtal membranes are homologous structures throughout the Amniotan series (also in my view a perfectly justifiable assumption)[1], then the homologies of this extra-embryonal region of the Marsupial blastocyt are not far to seek. It is clearly the homologue of the extra-embryonal ectoderm of the Sauropsidan and Monotreme egg, and the homologue also of the outer enveloping layer of the Eutherian blastocyst, to which Hubrecht has given the special name of "trophoblast." In my view the trophoblast is none other than extra-embryonal ectoderm which in the viviparous mammals, in correlation with the intra-uterine mode of development, has acquired a special significance for the nutrition of the embryo.

These, then, are my conclusions, and to me they seem on general grounds perfectly obvious, viz.: (1) that the embryonal or formative region of the unilaminar Marsupial blastocyst is the homologue of the inner cell-mass or

[1] How Assheton can maintain ('09, p. 266) "that the amnion of the rabbit is not more homologous to the amnion of the Sauropsidan than the horny teeth of Ornithorhynchus are homologous to the true teeth of the mammal or reptile, which they have supplanted," how he can hold this view and yet proceed to utilise the presence of the amnion as one of the leading characters distinguishing the Amniota from the Anamnia, I fail to comprehend. Surely the presence of a series of purely analogous structures in a group is of no classificatory value.

Table of Comparison of the Early Ontogeny in the Three Mammalian Sub-classes.

	Prototheria.	Metatheria.	Eutheria.
Secondary egg-envelopes	Shell	Shell-membrane	No shell-membrane.
	Albumen	Albumen	Albumen (in some forms).
	Zona	Zona	Zona.
Ovum	Large for mammalia (3·5–4 mm. in diameter), telolecithal with discrete yolk-spheres, like those of Sauropsida.	Minute compared with that of Prototheria, but larger than average Eutherian ovum (·24 mm. in diameter in Dasyurus), telolecithal in type, but becoming secondarily homolecithal as result of elimination of surplus deutoplasmic material.	Minute (varying from ·07 mm. in mouse to ·2 mm. in man), with, in some forms, polar differentiation of its formative and deutoplasmic materials.
Cleavage	Meroblastic, at first radial, blastomeres of 4-celled stage being radially arranged around polar diameter; results in formation of a several-layered cleavage-disc which gives origin to a unilaminar blastodermic membrane (Semon), composed of embryonal and extra-embryonal regions, though no clear line of separation between them has as yet been recognised.	Secondarily holoblastic and of radial type; blastomeres of 4-celled stage radially arranged and enclosing segmentation cavity. Third cleavages meridional like the first two; in absence of yolk-mass the resulting eight blastomeres forming an open ring. Fourth cleavages equatorial, unequal and qualitative, with resulting formation of two superimposed cell-rings, respectively formative (embryonal) and non-formative (extra-embryonal) in significance.	Secondarily holoblastic; blastomeres of 4-celled stage, in absence of yolk-mass and shell, not arranged radially but in two pairs at right angles to each other, thus forming a cross-shaped group. Further divisions result in formation of solid morula, composed of two predetermined groups of cells, homologous with the formative and non-formative cell-rings of the Metatherian.

	Prototheria.	Metatheria.	Eutheria.
Blasto-cyst	Formed directly by the peripheral growth of the extra-embryonal region of the unilaminar blastodermic membrane, in contact with inner surface of zona. Blastocyst cavity represents subgerminal cavity extended by liquefaction so as to include entire yolk-mass.	Formed directly by the spreading towards opposite poles, of the products of division of the two cell-rings of the 16-celled stage in contact with inner surface of the sphere constituted by the egg-envelopes. Blastocyst cavity represents the persistent segmentation cavity.	Formed indirectly as result of complete envelopment of the formative by the non-formative cells in the morula stage, and subsequent formation of blastocyst cavity by the confluence of inter- or intra-cellular vacuolar spaces, either or both.
—	Embryonal region of unilaminar blastodermic membrane freely exposed and superficial, there being no evidence of the existence at any period during early stages of development of a cellular layer externally to it. The entypic condition therefore does not occur.	Unilaminar wall of blastocyst formed conjointly by the formative (embryonal) and non-formative (extra-embryonal) regions; formative region from the first superficial. The entypic condition therefore does not occur.	Unilaminar wall of blastocyst formed exclusively by the non-formative (tropho-ectodermal) cells, the formative cells of the embryonal knot being completely enclosed, i.e. the entypic condition occurs.
—	Embryonal region of unilaminar blastodermic membrane probably gives origin to parent cells of entoderm by a process of proliferation. At an early period before blastocyst wall is completed (?). Extra-embryonal region of same directly forms the extra-embryonal ectoderm.	Formative region of blastocyst wall furnishes the embryonal ectoderm and the entire entoderm of the blastocyst. Non-formative region forms directly the outer layer of the chorion and omphalopleure of later stages, and is therefore homologous with the extra-embryonal ectoderm of the Prototheria.	The embryonal knot furnishes the embryonal ectoderm and the entire entoderm of the vesicle, and is therefore homologous with the formative region of Marsupial blastocyst. The enveloping layer of non-formative cells forms the outer layer of the omphalopleure and chorion of later stages, and is therefore the homologue of the non-formative region of the Marsupial blastocyst and of the extra-embryonal ectoderm of the Monotreme.

embryonal knot of the Eutherian blastocyst; and (2) that the extra-embryonal or non-formative region of the same is the homologue of the extra-embryonal ectoderm of the Sauropsida and Monotremata and of the trophoblast of the Eutheria.

As regards conclusion (1) there is not likely to be much difference of opinion, but as regards (2), whilst perhaps the majority of embryologists support the obvious, not to say common-place view which I here advocate, it seems certain that it will prove neither obvious nor acceptable to those mammalian embryologists (I refer specifically to my friends Professor A. A. W. Hubrecht and Mr. R. Assheton) who, with only Selenka's account of early Marsupial ontogeny before them, have formulated other and quite divergent views as to the morphological nature of the outer enveloping layer of the Eutherian blastocyst. It is therefore necessary to discuss this question further, though I would fain express my conviction that had the observations recorded in this paper been earlier available, much vain speculation as to the phylogeny of the trophoblast might possibly have been avoided.

Chapter VII.—The Early Ontogeny of the Mammalia in the Light of the Foregoing Observations.

In entering on a discussion of the bearings of the results of my study of the early development of Marsupials on current interpretations of early Mammalian ontogeny, and especially of the homologies of the germ-layers, I desire at the outset to emphasise my conviction that, specialised though the Marsupials undoubtedly are in certain features of their anatomy, e. g. their dentition, genital ducts, and mammary apparatus, the observations recorded in the preceding pages of this paper afford not the slightest ground for the supposition that their early ontogeny is also of an aberrant type, devoid of significance from the point of view of that of other mammals. On the contrary, I hope to demonstrate that the Marsupial type of early development not only readily

falls into line with that of Eutheria, and with what we know of the early development of the Prototheria, but furnishes us with the key to the correct interpretation of that extraordinarily specialised developmental stage, the Eutherian blastocyst. In particular I hope to show that the description which I have been able to give of the mode of formation of the Marsupial blastocyst, bridges in the most satisfactory fashion the great gap which has till now existed in our knowledge of the way in which the transition from the Monotrematous to the Eutherian type of development has been effected.

I. The Early Development of the Monotremata.

Our knowledge of the early development of the oviparous mammals is admittedly still far from complete. Nevertheless it is not so absolutely fragmentary that it can be passed over in any general discussion of early mammalian ontogeny, and I certainly cannot agree with the opinion of Assheton ('08, p. 227) that from it "we gain very little help towards the elucidation of Eutherian development." On the contrary, I think that the combined observations of Semon ('94), and Wilson and Hill ('07) shed most valuable light on the early ontogenetic phenomena in both the Metatheria and Eutheria. I propose therefore to give here a very brief resumé of the chief results of these observers,[1] and at the same time to indicate how the knowledge of early Monotreme ontogeny we possess, limited though it be, does help us to a better understanding of the phenomena to which I have just referred.

The ovum, as is well known from the observations of Caldwell ('87), is Reptilian in its character in all but size. It is yolk-laden and telolecithal, the yolk consisting of discrete yolk-spheres, and it is enclosed outside the zona (vitelline membrane) by a layer of albumen and a definite shell.

[1] In so doing I have largely utilised the phraseology of Wilson and Hill's paper ('07).

At the moment of entering the oviduct it has a diameter of 3·5–4 mm. (2·5–3 mm. according to Caldwell), and is therefore small relatively to that of a reptile of the same size as the adult Monotreme, but large relatively to those of other mammals, being about twelve times larger than that of Dasyurus, and about eighteen times larger than that of the rabbit.

Cleavage is meroblastic. The first two cleavage planes are at right angles to each other, as in the Marsupial, and divide the germinal disc into four approximately equal-sized cells (Semon, Taf. ix, fig. 30). Each of these then becomes subdivided by a meridional furrow into two, so that an 8-celled stage is produced, the blastomeres being arranged symmetrically, or almost symmetrically, on either side of a median line, perhaps corresponding to the primary furrow (Wilson and Hill, p. 37, text-figs. 1 and 2). Imagine the yolk removed and the blastomeres arranged radially, and we have at once the open ring-shaped 8-celled stage of Dasyurus. The details of the succeeding cleavages are unknown. Semon has described a stage of about twenty-four cells (Semon, Taf. ix, fig. 31), in which the latter formed a one-layered circular plate with no evidence of bilateral symmetry, and this is succeeded by a stage also figured by Semon (figs. 32 and 33, cf. also Wilson and Hill, Pl. 2, fig. 2), in which the blastoderm has become several cells thick, though it has not yet increased in surface extent. It is bi-convex lens-shaped in section, its lower surface being sharply limited from the underlying white yolk. No nuclei are recognisable in the latter, either in this or any subsequent stage, nor is there ever any trace of a syncytial germ-wall, features in which the Monotreme egg differs from the Sauropsidan.

The next available stage, represented by an egg of Ornithorhynchus, described by Wilson and Hill ('07, p. 38, Pl. 2, fig. 4), and by an egg of Echidna, described by Semon ('94, p. 69, figs. 22 and 33), is separated by a considerable gap from the preceding, and most unfortunately so, since it belongs to the period of commencing formation of the germ-layers. The

cellular lens-shaped blastoderm of the preceding stage has now extended in the peripheral direction so as to enclose about the upper half of the yolk-mass, and in so doing it has assumed the form, almost exclusively, of a unilaminar thin cell-membrane, composed of flattened cells and closely applied to the inner surface of the zona. At the embryonic pole, however, in the region of the white yolk-bed, there are present in the Ornithorhynchus egg a few plump cells, immediately subjacent to the unilaminar blastoderm, but separate and distinct from it, whilst in the Echidna egg Semon's figure (fig. 33), which is perhaps somewhat schematic, shows a group of scattered cells, similar to those in the Ornithorhynchus egg but placed considerably deeper in the white yolk-bed. Unfortunately we have no definite evidence as to the significance of these internally situated cells. One of two possible interpretations may be assigned to them. Either they represent the last remaining deeply placed cells of the blastodisc of the preceding stage, which have not yet become intercalated in the unilaminar blastodermic membrane believed by Semon to be the condition attained in eggs of about this stage of development, or they are cells which have been proliferated off from this unilaminar blastoderm, to constitute the parent cells of the future yolk-entoderm. As regards Echidna, Semon expresses a definite enough opinion; he holds that these deeply placed cells actually arise by a somewhat diffuse proliferation or ingrowth from a localised depressed area of the blastoderm at the embryonic pole, and that they give origin to yolk-entoderm. This interpretation of Semon seems probable enough in view of the mode of origin of the entoderm in the Metatheria and Eutheria. Moreover in the next available stage, an egg of Ornithorhynchus, just over 6 mm. in diameter, described by Wilson and Hill, the blastoderm is already bilaminar throughout its extent, so that we might very well expect to find the beginnings of the entoderm in the somewhat younger eggs.

In the 6 mm. egg just referred to, the peripheral portion of the unilaminar blastoderm of the preceding stage has grown

so as to enclose the entire yolk-mass in a complete ectodermal envelope, whilst internally to that a complete lining of yolk-entoderm has become established. As the result of these changes, and of the imbibition of fluid from the uterus, the solid yolk-laden egg has become converted into a relatively thin-walled vesicle or blastocyst, possessed of a bilaminar wall surrounding the partly fluid vitelline contents of the egg. Throughout the greater part of its extent the structure of the vesicle wall is very simple. It consists externally of an extremely attenuated ectodermal cell-membrane closely adherent to the deep surface of the vitelline membrane (zona), and within that of a layer of yolk-entoderm, composed of large swollen cells, containing each a vesicular nucleus, and a number of yolk-spheres of varying size. Over a small area, overlying the white yolk-bed, however, the ectodermal layer of the wall presents a different character to that described above. Its constituent cells are here not flattened and attenuated, but irregularly cuboidal in form and much more closely packed together; moreover they stand in proliferative continuity with a subjacent mass of cells, also in process of division. The irregular superficial layer and this latter mass together form a thickened lenticular cake, ·5 mm. in greatest diameter, projecting towards the white yolk-bed but separated from it by the yolk-entoderm, which retains its character as a continuous cell-membrane. This differentiated, thickened area of the wall, situated as it is at the upper pole of the egg, as marked by the white yolk-bed, must be held to represent a part of the future embryonal region. Wilson and Hill incline to regard it as in some degree the equivalent of the "primitive plate" of Reptiles and as the initial stage in the formation of the primitive knot of later eggs. This question, however, does not closely concern us here: the point I wish to emphasise is the relative inactivity of the cells composing the embryonal region of the blastoderm in the Monotreme as compared with the marked activity displayed by those constituting the peripheral (extra-embryonal) region of the same. It is these latter cells which by their

rapid growth complete the envelopment of the yolk-mass and so constitute the lower hemisphere of the blastocyst.

The bilaminar blastocyst of the Monotreme, formed in the manner indicated above, is entirely comparable with the Marsupial blastocyst of the same developmental stage. There are differences in detail certainly (e.g. in the characters, time of formation, and rate of spreading of the entoderm, in the mode of formation of the blastocyst cavity and in its contents, in the apparent absence in the Monotreme of any well-marked line of division between the embryonal and extra-embryonal regions of the ectoderm, in the relatively earlier appearance of differentiation in the embryonal region in the Monotreme as compared with the Marsupial), but the agreements are obvious and fundamental; in particular, I would emphasise the fact that in both the embryonal region is superficial and freely exposed, and forms part of the blastocyst wall just as that of the reptile forms part of the general blastoderm. Moreover, should future observations confirm the view of Semon that the primitive entodermal cells of the Monotreme are proliferated off from the embryonal region of the unilaminar blastoderm, then we should be justified in directly comparing the latter with the unilaminar wall of the Marsupial blastocyst, and in regarding it also as consisting of two differentiated regions, viz. a formative or embryonal region, overlying the white yolk-bed, and giving origin to the embryonal ectoderm and the yolk-entoderm, and a non-formative region which rapidly overgrows the yolk-mass so as to eventually completely enclose it, just as does the less rapidly growing extra-embryonal ectoderm of the Sauropsidan blastoderm.[1] Meantime I see no reason for doubting that this rapidly growing peripheral portion of the unilaminar blastoderm of the Monotreme is anything else than extra-embryonal ectoderm homogenous with that of the reptile. Indeed, I am not aware that any embryologist except Hubrecht thinks otherwise. Even Assheton is, I believe, content to

[1] We should further be justified in concluding that the entoderm is similar in its mode of origin in all three mammalian sub-classes.

regard the outer layer of the Monotreme blastocyst as ectodermal. Hubrecht's view is that the primitive entodermal cells of Semon give origin, not to yolk-entoderm, but to the equivalent of the embryonal knot of Eutheria, whilst the unilaminar blastodermic membrane itself is a larval layer —the trophoblast—that portion of it overlying the internally situated cells representing the covering layer (Rauber's layer) of the Eutherian blastocyst. "For this view," remarks Assheton ['09, p. 233), "I can see no reason derivable from actual specimens described and figured by those four authors" (Caldwell, Semon, Wilson and Hill), with which criticism I am in entire agreement, as also with the following statement, which, so far as the Metatheria are concerned, is based on my own results: "Neither in the Prototheria [n] or the Metatheria is there really any tangible evidence of a trophoblast occurring as a covering layer over the definitive epiblast as in Eutheria" (p. 234).

In connection with the peripheral growth of the unilaminar blastoderm in the Monotreme, it is of interest to observe that this takes place, not apparently in intimate contact with the surface of the solid yolk, as is the case with the growing margin of the extra-embryonal ectoderm in the Sauropsidan egg, but rather in contact with the inner surface of the thickened zona, perhaps as the result of the accumulation in the perivitelline space of fluid which has diffused into the latter from the uterus. In other words, the peripheral growth of the extra-embryonal ectoderm to enclose the yolk-mass appears to take place here in precisely the same way as the spreading of the non-formative cells in Dasyurus to complete the lower pole of the blastocyst. In my view the latter phenomenon is none other than a recapitulation of the former; on the other hand, I regard the spreading of the formative cells in Dasyurus towards the upper pole as a purely secondary feature, conditioned by the loss of the yolk-mass and the attainment of the holoblastic type of cleavage.

If it be admitted that the outer extra-embryonal layer of the Monotreme blastocyst is homogenous with the extra-

embryonal ectoderm of the Reptile, then it seems to me there is no escape from the conclusion that these layers are also homogenous with the non-formative region of the unilaminar Marsupial blastocyst. I need only point out here that the chief destiny of each of the mentioned layers, and I might also add that of the outer enveloping layer of the Eutherian blastocyst (the so-called trophoblast), is one and the same, viz. to form the outer layer of the chorion (false amnion, serous membrane) and omphalopleure (unsplit yolk-sac wall, Hill ['97]),[1] and that to deny their homogeny to each other implies the non-homogeny of these membranes and the amnion in the Amniotan series, and consequently renders the group name Amniota void of all morphological meaning.

The rapidity with which the enclosure of the yolk-mass is effected, and the relative tardiness of differentiation in the embryonal region are features which sharply distinguish the early ontogeny of the Monotremes from that of the Sauropsida, and which, in my view, are of the very greatest importance, since they afford the key to a correct understanding of the peculiar cœnogenetic modifications observable in the early ontogeny of the Metatheria and Eutheria. To appreciate the significance of these features it is necessary to take account of the great difference which exists between the Sauropsidan and Monotreme ovum in regard to size, as well as of the very different conditions under which the early development goes on in the two groups. The Sauropsidan egg is large enough to contain within its own confines the amount of yolk necessary for the production of a young one complete in all its parts and capable of leading an independent existence immediately it leaves the shell. Furthermore, it is also large

[1] In certain Amniotes the layers in question appear also to participate in the formation of the inner lining of the amnion (amniotic ectoderm) (cf. Assheton ['09], pp. 248–9), but this does not affect the statement in the text. In the Sauropsida and Monotremata I think I am correct in saying that no sharp distinction is recognisable between the embryonal and extra-embryonal regions of the ectoderm, hence it is difficult, if not impossible, to determine with certainty their relative participation in the formation of the amniotic ectoderm.

enough to provide room for the development of an embryo without any secondary growth in size after it leaves the ovary. Moreover we have to remember that after it has become enclosed in the shell, it remains but a short time in the oviduct and receives little or no additional nutrient material from the oviducal walls. The yolk-mass in any case retains its solid character; there is no necessity for its rapid enclosure, and so enclosure is effected slowly, contemporaneously with the differentiation of the embryo.

In the Monotreme the conditions are altogether different. The ripe ovarian ovum when it enters the oviduct has a diameter of about 3·5 to 4 mm., and is thus considerably smaller than that of a Reptile of the same size as the adult Monotreme. The amount of yolk which it is capable of containing is not anything like sufficient to last the embryo throughout the developmental period, and, moreover, it does not provide the space essential for the development of an embryo on the ancestral Reptilian lines. As Assheton ('98, p. 251) has pointed out, "the difference in size between the fertilised ovum of a reptile or bird or of a mammal is very great; but the difference in size between the embryo of, say, a bird with one pair of mesoblastic somites and of a mammal of the same age is comparatively small. This means that nearly the same space is required for the production of the mammalian embryo as of the Sauropsidan, and has to be provided." In the Monotreme not only is additional room necessary, but also additional nutrient material, sufficient with that already present in the egg to last the embryo throughout the period of incubation. Both are acquired contemporaneously during the sojourn of the egg in the uterine portion of the oviduct, wherein the egg increases greatly in size. When it enters the uterus, the Monotreme egg has a diameter, inclusive of its membranes, of about 4–5 mm.; when it is laid, it measures in Ornithorhynchus, in its greatest diameter, 16–19 mm., and somewhat less in the case of Echidna. Prior to the enclosure of the yolk the increase in diameter, due to the accumulation of fluid in

the perivitelline space and between the zona and shell, is but slight. But as soon as the yolk becomes surrounded by a complete cellular membrane, i. e. as soon as the egg has become converted into a thin-walled blastocyst, rapid growth sets in, accompanied by the active imbibition of the nutrient fluid, which is poured into the uterine lumen as the result of the secretory activity of the abundantly developed uterine glands. The fluid absorbed not only keeps the blastocyst turgid, but it brings about the more or less complete disintegration of the yolk-mass, its constituent spherules becoming disseminated in the fluid contents of the blastocyst cavity. Although a distinct and continuous subgerminal cavity, such as appears beneath the embryonal region of the Sauropsidan blastoderm, does not occur in the Monotreme egg, vacuolar spaces filled with fluid develop in the white yolk-bed underlying the site of the germinal disc and appear to represent it. As Wilson and Hill remark ('03, p. 317), "one can, without hesitation, homologise the interior of the vesicle with the subgerminal cavity of a Sauropsidan egg, extended so as to include by liquefaction the whole of the yolk itself." In the Marsupial the blastocyst cavity has a quite different origin, since it represents the persistent segmentation cavity, whilst in the Eutheria the same cavity is secondarily formed by the confluence of intra- or inter-cellular vacuolar spaces, but no one, so far as I know, has ever ventured to assert that, because of this difference in mode of origin, the blastocyst cavity in the series of the Mammalia is a non-homogenous formation.

To return to the matter under discussion, it appears to me that the necessity which has arisen, consequent on the reduction in size of the ovum, for rapid growth of the same in order to provide room for the development of an embryo and for the storage of nutrient material furnished by the maternal uterus, affords a satisfactory explanation of the much more marked activity of the extra-embryonal region of the blastoderm as compared with the embryonal, which is such a striking feature in the early ontogeny of the Monotremes, and not

only of them, but, as Assheton has pointed out ('98, p. 251), of the higher mammals as well (cf. the process of epiboly and the inertness at first displayed by the formative cells of the embryonal knot as compared with the activity of the non-formative or tropho-ectodermal cells), an activity which results in the rapid completion of that characteristically mammalian developmental stage—the blastocyst or blasto-dermic vesicle.

The necessity for the early formation of such a stage, capable of rapidly growing in a nutrient fluid medium provided by the mother, has profoundly influenced the early ontogeny in all three mammalian subclasses, and naturally most of all that of the Eutheria, in which reduction of the ovum, both as regards size and secondary envelopes, has reached the maximum. And I think there can be little doubt but that it is this necessity which has induced that early separation of the blastomeres into two categories, respectively formative and non-formative in significance, which has long been recognised as occurring in Eutheria, and which I have shown also occurs amongst the Metatheria. This early separation of the blastomeres into two distinct groups is not recognisable in the Sauropsida, and the idea that it is in some way connected with the loss of yolk which the mammalian ovum has suffered in the course of phylogeny, was first put forward, I believe, by Jenkinson. In his paper on the germinal layers of Vertebrata ('06, p. 51) he writes: "Segmentation therefore is followed in the Placentalia by the separation of the elements of the trophoblast from those destined to give rise to the embryo and the remainder of its fœtal membranes, and this 'precocious segregation' seems to have occurred phylogenetically during the gradual loss of yolk which the egg of these mammals has undergone." Whether or not such a "precocious segregation" has already become fixed in the Monotremes, future investigation must decide (cf. ante, p. 90).

The loss of yolk, with resulting reduction in size which the Monotreme ovum has suffered in the course of phylogeny, we

must assume to have taken place gradually and in correlation with the longer retention of the egg in the oviduct, the elaboration of the uterine portion of the same as an actively secretory organ, and the evolution of the mammary apparatus. The Monotremes thus render concrete to us one of the first great steps in mammalian evolution so far as developmental processes are concerned, viz. the substitution for intra-ovular yolk of nutrient material furnished directly by the mother to the developing egg or embryo. We see in them the beginnings of that process of substitution of uterine for ovarian nutriment which reaches its culmination in the Eutheria with their microscopic yolk-poor ova and long intra-uterine period of development. The Marsupials show us in Dasyurus an interesting intervening stage so far as the ovum is concerned, in that this, though greatly reduced as compared with that of the Monotreme, still retains somewhat of its old tendencies and elaborates more yolk-material than it can conveniently utilise, with the result that it has to eliminate the surplus before cleavage begins. But as concerns their utilisation of intra-uterine nutriment, they have specialised along their own lines, and instead of exhausting the possibilities implied by the presence of that, they have extensively elaborated the mammary apparatus for the nutrition of the young, born in a relatively immature state, after a short period of intra-uterine life (cf. Wilson and Hill ['97, p. 580]).

In view of the fact that the young Monotreme enjoys three developmental periods, viz. intra-uterine, incubatory, and lactatory, the question might be worthy of consideration whether it may not be that the Marsupial has merged the incubatory period in the lactatory, the Entherian the same in the intra-uterine.

2. The Early Development of the Metatheria and Eutheria.

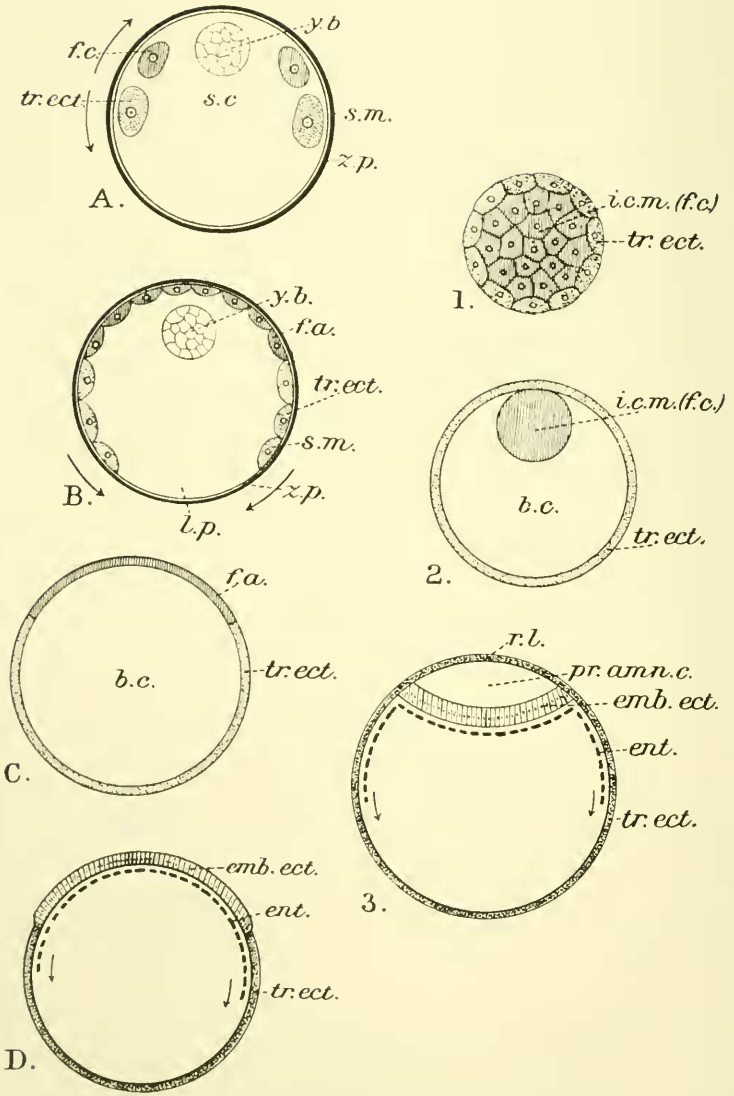
It will have become evident from the foregoing that the Metatherian mode of early development is to be regarded as

but a slightly modified version of the Prototherian, such differences as exist between them being interpretable as cœnogenetic modifications, induced in the Metatherian by the practically complete substitution of uterine nutriment for intra-ovular yolk, a substitution which has resulted in the attainment by the marsupial ovum of the holoblastic type of cleavage. In the present section I hope to demonstrate how the early ontogeny of the Metatheria enables us to interpret that of the Eutheria in terms of that of the Prototheria.

If we proceed to compare the early development in the Metatheria and Eutheria, we encounter, from the 4-celled stage onwards, such obvious and profound differences in the mode of formation of the blastocyst, and in the relations of its constituent parts, that the differences seem at first sight to far outweigh the resemblances. Nevertheless, apart from their common possession of the same holoblastic mode of cleavage, there exists one most striking and fundamental agreement between the two in the fact that in both there occurs, sooner or later during the cleavage process, a separation of the blastomeres into two distinct, pre-determined cell-groups, whose individual destinies are very different, but apparently identical in the two subclasses. In the Marsupial, as typified by Dasyurus, the fourth cleavages are, as we have seen, unequal and qualitative, and result in the separation of two differentiated groups of blastomeres, arranged in two superimposed rings, viz. an upper ring of eight smaller, less yolk-rich cells, and a lower of eight larger, more yolk-rich cells. The evidence justifies the conclusion that the former gives origin directly to the formative or embryonal region of the vesicle wall, the latter to the non-formative or extra-embryonal region.

Amongst the Eutheria the evidence is no less clear. It has been conclusively shown by various observers (Van Beneden, Duval, Assheton, Hubrecht, Heape, and others) that, sooner or later, there occurs a separation of the blastomeres into two distinct groups, one of which eventually encloses the other completely. The two groups may be clearly distinguishable

TEXT-FIG. 2.



Diagrams illustrating the mode of formation of the blastocyst in Metatheria (A–D) and Eutheria (1–3). *b.c.* Blastocyst cavity. *i.c.m.* Inner cell-mass. *pr.amn.c.* Primitive amniotic cavity. *r.l.* Rauber's layer. *s.c.* Segmentation cavity. For other reference letters see explanation of plates (p. 125).

in early cleavage stages, owing to differences in the characters and staining reactions of their cells, and in such cases there is definite evidence of the occurrence of a process of overgrowth or epiboly, whereby one group gradually grows round and completely envelops the other, so that in the completed morula a distinction may be drawn between a central cell-mass and a peripheral or enveloping layer (rabbit, Van Beneden; sheep, Assheton). In other cases, where it has been impossible to recognise the existence of these two distinct cell-groups in the cleavage stages, we nevertheless find, either in the completed morula or in the blastocyst, that a more or less sharp distinction may be drawn between an enveloping layer of cells and an internally situated cell-mass (inner cell-mass).

E. van Beneden, in his classical paper on the development of the rabbit, published in 1875, was the first to recognise definitely the existence of two categories of cells in the segmenting egg of the Eutherian mammal. In this form he showed how in the morula stage a cap of lighter blastomeres gradually grows round and envelops a mass of more opaque cells by a process of overgrowth or epiboly. In his more recent and extremely valuable paper on the development of Vespertilio ('99), he again demonstrated the existence of two groups of blastomeres as well in the segmenting egg as in the completed morula, but failed to find evidence of epiboly in all cases. Nevertheless he holds fast to the opinion which he expressed in 1875: "Que la segmentation s'accompagne, chez les Mammifères placentaires, d'un enveloppement progressif d'une partie des blastomères par une couche cellulaire, qui commence à se différencier dès le début du développement," and states that "dans tous les œufs arrivés à la fin de la segmentation et dans ceux qui montraient le début de la cavité Blastodermique j'ai constamment rencontré une couche périphérique complète, entourant de toutes parts un amas cellulaire interne, bien séparé de la couche enveloppante." The latter layer he regards as corresponding to the extra-embryonal ectoderm of the Sauropsida, and points out that

"chez tous les Chordés les premiers blastomères qui se differencient et qui avoisinent le pôle animal de l'œuf sont des éléments épiblastiques. C'est par la couche cellulaire qui résulte de la segmentation ultérieure de ces premiers blastomères épiblastiques que se fait, chez les Sauropsides, l'enveloppement du vitellus. Dans l'œuf réduit à n'être plus qu'une sphère microscopique, l'épibolie a pu s'achever dès la fin de la segmentation, voire même avant l'achèvement de ce phénomène." The "amas cellulaire interne" (embryonal knot, inner cell mass), Van Beneden shows, differentiates secondarily into "un lécithophore et un bouton embryonnaire." The former is the entoderm of other authors, the latter the formative or embryonal ectoderm. Hubrecht, in the forms studied by him (Sorex, Tupaia, Tarsius[1]) finds a corresponding differentiation. In Tupaia he describes the morula stage as consisting of a single central lightly staining cell, which he regards as the parent cell of the inner cell-mass of later stages, and of a more darkly staining peripheral layer which forms the unilaminar wall of the blastocyst. Here, then, the parent cells of the two cell-groups would appear to be separated at the first cleavage. Hubrecht, like Van Beneden, holds that the inner cell-mass furnishes the embryonal ectoderm and the entire entoderm of the blastocyst. The peripheral layer he has termed the trophoblast ('88, p. 511), and in his paper on the placentation of the hedgehog ('89, p. 298) he defines the term as follows: "I propose to confer this name to the epiblast of the blastocyst as far as it has a direct nutritive significance, as indicated by proliferating processes, by immediate contact with maternal tissue, maternal blood, or secreted material. The epiblast of the germinal area—the formative epiblast—and that which will take part in the formation of the inner lining of the amnion cavity is, ipso facto, excluded from the definition." Thus the name

[1] In Erinaceus the entoderm, from Hubrecht's observations, appears to be precociously differentiated, prior to the separation of the embryonal ectoderm from the overlying trophoblast, but the details of the early development in this form are as yet only incompletely known.

trophoblast was originally employed by Hubrecht as a convenient term designatory of what he at the time regarded as the extra-embryonal ectoderm of the mammalian blastocyst. In the course of his speculations on the origin of this layer, however, he has reached the conclusion that it is really of the nature of "a larval envelope, an Embryonalhülle" ('08, p. 15), inherited by the mammals, not from the reptiles (which have no direct phylogenetic relationship to the latter), but from their remote invertebrate ancestors ("vermiform predecessors of cœlenterate pedigree, provided with an ectodermal larval investment [Larvenhülle]").

Assheton, again, although he was unable to convince himself ('94) of the correctness of van Beneden's account of the occurrence of a process of epiboly in the segmenting eggs of the rabbit, finds in the sheep ('98) that a differentiation into two groups of cells is recognisable "perhaps as early as the eight segment stage," and that one of the groups gradually envelops the other. "Let it be noted," he writes ('98, p. 227), "that we have now to face the fact, based on actual sections, that there is in certain mammals a clear separation of segments at an early stage into two groups, one of which eventually completely surrounds the other," and instances Van Beneden's observations on the rabbit (of the correctness of which he, however, failed to satisfy himself, as noted above), Duval's observations on the bat, Hubrecht's on Tupaia, and his own on the sheep. Assheton thinks this phenomenon "must surely have some most profound significance," but finds himself unable to accept the interpretations of either Van Beneden or Hubrecht, and puts forward yet another view, "based on the appearance of some segmenting eggs of the sheep" ('08, p. 233), "that in cases where this differentiation does clearly occur, it is a division into epiblast and hypoblast, the latter being the external layer" ('98, p. 227). Assheton thus differs from all other observers in holding that the inner cell-mass or embryonal knot of the Eutherian blastocyst gives origin solely to the formative or embryonal ectoderm, and I believe I am correct in stating that he also

differs from all other observers in holding that the outer enveloping layer of the same is entodermal.[1]

The fact, then, of the occurrence amongst Eutheria of a "precocious segregation" of the blastomeres into two distinct groups, one of which eventually surrounds the other completely, is not in dispute, though authorities differ widely in the interpretation they place upon it. In the Eutherian blastocyst stage, the enveloping layer forms the outer unilaminar wall of the vesicle, and encloses the blastocyst cavity as well as the other internally situated group. This latter typically appears as a rounded cell-mass, attached at one spot to the inner surface of the enveloping layer, but more or less distinctly marked off from it. It is generally termed the inner cell-mass or embryonal knot ("amas cellulaire interne" of Van Beneden). For the enveloping layer Hubrecht's name of "trophoblast" is now generally employed, even by those who refuse to adopt the speculative views with which its originator has most unfortunately, as I think, enshrouded this convenient term.

I have demonstrated the occurrence of an apparently comparable "precocious segregation" of the blastomeres into two distinct groups in one member of the Metatheria which there is no reason to regard as an aberrant type, and I have shown beyond all shadow of doubt that from the one group, which constitutes what I have termed the formative region of the unilaminar vesicle-wall, there arise the embryonal ectoderm and the entire entoderm of the vesicle, both embryonal and extra-embryonal, and that the other group, which constitutes the non-formative region of the vesicle-wall, directly furnishes the extra-embryonal ectoderm, i. e. the ectoderm of the omphalopleure and chorion.[2]

[1] Assheton states ('08, p. 233, cf. also '98, p. 220) that his interpretation "owes much also to the theoretical conclusions of Minot and Robinson." However that may be, both Minot and Robinson in their most recent writings continue to speak of the chorionic ectoderm.

[2] Whether or not it participates in the formation of the amniotic ectoderm future investigation must decide.

As regards Eutheria, we have seen that Van Beneden and Hubrecht, though their views in other respects are widely divergent, both agree that the inner cell-mass of the blastocyst furnishes the embryonal ectoderm (as well as the amniotic ectoderm wholly or in part) and the entire entoderm of the vesicle. That, in fact, is the view of Mammalian embryologists generally (Duval and Assheton excepted),[1] and if we may assume it to be correct, then it would appear that the later history of the formative region of the Marsupial blastocyst and that of the inner cell-mass of the Eutherian are identical. That being so, and bearing in mind that both have been shown, at all events in certain Mammals, to have an identical origin as a group of precociously segregated blastomeres,[2] I can come to no other conclusion than that they are homogenous formations. If that be accepted, then this fact by itself renders highly probable the view that the so-called trophoblast of the Eutherian blastocyst is homogenous with the non-formative region of the Metatherian vesicle, and when we reflect that both have precisely the same structural and topographical (not to mention functional) relations in later stages, inasmuch as they constitute the ectoderm of the chorion and omphalopleure (with or without participation in the formation of the amniotic ectoderm), and that both have a similar origin in those Mammals in which a precocious segregation of the blastomeres has been recognised, their exact

[1] The view of Duval ['95], based on the study of Vespertilio, that the inner cell-mass gives rise solely to entoderm, and that the enveloping layer furnishes not only the extra-embryonal but also the embryonal ectoderm, is shown by Van Beneden's observations on the same form to be devoid of any basis of fact. Assheton's views are referred to below (p. 110).

[2] The fact that the phenomenon of the "precocious segregation" of the blastomeres into two groups with determinate destinies has already become fixed in the Marsupial lends additional weight to the view of Van Beneden that such a segregation will eventually be recognised as occurring in all Eutheria without exception. Without it, it is difficult to understand how the entypic condition, characteristic of the blastocysts of all known Eutheria, is attained, unless by differentiation in situ, which seems to me highly improbable.

homology need no longer be doubted. In the preceding section of this paper (ante, pp. 91, 92) I have shown reason for the conclusion that the non-formative region of the Marsupial blastocyst is the homologue of the extra-embryonal ectoderm of the Monotreme and Reptile, and if that conclusion be accepted it follows that the outer enveloping layer of the Eutherian blastocyst, the so-called trophoblast of Hubrecht, is none other than extra-embryonal ectoderm, as maintained by Van Beneden, Keibel, Bonnet, Jenkinson, Lee, MacBride and others, the homologue of that of Reptilia.

I am therefore wholly unable to accept the highly speculative conclusions of Hubrecht, set forth with such brilliancy in a comparatively recent number of this Journal ('08), as to the significance and phylogeny of this layer. These conclusions, on the basis of which he has proceeded to formulate such far-reaching and, indeed, revolutionary ideas not only on questions embryological, but on those pertaining to the phylogeny and classification of vertebrates, have already been critically considered by Assheton ('09) and MacBride ('09), also in the pages of this Journal, and found wanting, and they are, to my mind, quite irreconcilable with the facts I have brought to light in regard to the early development of Marsupials. I yield to no one in my admiration for the epoch-making work of Hubrecht on the early ontogeny and placentation of the Mammalia, and I heartily associate myself with the eulogium thereanent so admirably expressed by Assheton in the critique just referred to (p. 274), but I am bound to confess that as concerns his views on the phylogeny of this layer, which he has termed the "trophoblast," he seems to me to have forsaken the fertile field of legitimate hypothesis for the barren waste of unprofitable speculation, and to have erected therein an imposing edifice on the very slenderest of foundations.

Before I proceed to justify this, my estimate of Hubrecht's views on the phylogeny of the trophoblast, let me first set forth his conception so far as I understand it. He starts with the assumption that the vertebrates (with the exception

of Amphioxus, the Cyclostomes, and the Elasmobranchs) are descended from "vermiform predecessors of cœlenterate pedigree" possessed of free-swimming larvæ, in which there was present a complete larval membrane of ectodermal derivation, and of the same order of differentiation "as the outer larval layer which in certain Nemertines, Gephyreans, and other worms often serves as a temporary envelope that is stripped off when the animal attains to a certain stage of development." When, for oviparity and larval development, viviparity and embryonic development became established in the Protetrapodous successors of the ancestral vermiform stock, the larval membrane did not disappear. On the contrary, it is assumed that it merely changed "its protective or locomotor function into an adhesive one," and so, development now taking place *in utero*, it is quite easy to understand how the larval membrane could gradually become transformed into a trophic vesicle, containing the embryo as before, and functional in the reception of nutriment from the walls of the maternal uterus. The final stages in the evolution of this trophic vesicle constituted by the old larval membrane are met with amongst the mammals, since in them it became vascularised so as to constitute a "yet more thorough system of nourishment at the expense of the maternal circulatory system." Such, then, is the phylogeny of the trophoblast according to Hubrecht. The Eutherian mammals, which it is held trace their descent straight back to some very early Protetrapodous stock, viviparous in habit and with small yolk-poor, holoblastic eggs, exhibit the trophoblast in its most perfect condition. Hubrecht therefore starts with them, and attempts to demonstrate the existence of a larval membrane, or remnants of such, externally to the embryonal ectoderm in all vertebrates with the exceptions already mentioned. There is no question of its existence in the Meta- and Eutherian mammals. "We may," writes Hubrecht ('08, p. 12), . . . "insist upon the fact that . . . all Didelphia and Monodelphia hitherto investigated show at a very early moment the didermic stage out of

which the embryo will be built up enclosed in a cellular vesicle (the trophoblast), of which no part ever enters into the embryonic organisation." The common possession by the Metatheria and Eutheria of a larval membrane is after all only what might be expected, "since after Hill's ('97) investigations, we must assume that the didelphian mammals are not descended from Ornithodelphia but from monodelphian placental ancestors." As concerns the Prototheria, although they cannot in any sense be regarded as directly ancestral to the other mammals, we nevertheless find the trophoblastic vesicle "comparatively distinct." "In many reptiles and birds," however, it is "distinguished with great difficulty from the embryonic shield," and this is explained by the fact that the Sauropsida which are assumed to have taken their origin from the same Protetrapodous stock as the mammals but along an entirely independent line, have secondarily acquired, like the Prototheria, the oviparous habit, with its concomitants, a yolk-laden egg and a shell, and this latter acquisition has naturally tended "to relegate any outer larval layer to the pension list" ('09, p. 5). "Concerning the yolk accumulation in the Sauropsidan egg, there is no trouble at all to suppose that the vesicular blastocyst of an early viviparous ancestor had gradually become yolk-laden. The contrary assumption, found in the handbooks, that the mammalian egg, while totally losing its yolk, has yet preserved the identical developmental features as the Sauropsid, is in reality much more difficult to reconcile with sound evolutionary principles" ('09, p. 5).

Amongst the lower Vertebrates the larval membrane is clearly enough recognisable in the so-called Deckschicht of the Teleostomes, Dipnoans, and Amphibians. It is frankly admitted that Amphioxus, the Cyclostomes, and the Elasmobranchs "show in their early development no traces of a Deckschicht" (larval layer, trophoblast), but there is no difficulty about this, since it is easy enough to suppose, in view of other characters, that "the Selachians may very well have descended from ancestors without any outer larval layer"

('08, p. 151), and "for Cyclostomes the same reasoning holds good" (p. 152).

The trophoblast, then, is conceived of by Hubrecht as a larval membrane of ectodermal derivation, which invests the embryonal anlage in all Vertebrates with the exceptions mentioned, which is subject to secondary reduction, and which is homologous throughout the series. As I understand the conception, what is ordinarily called extra-embryonal ectoderm in the Sauropsida is not trophoblast, otherwise Hubrecht could hardly write—"in reptiles and birds traces of the larval layer have in late years been unmistakably noticed" ('09, p. 5); nevertheless what other writers have termed embryonal and extra-embryonal ectoderm in the Prototheria is claimed by Hubrecht as trophoblast (at all events that is my interpretation of his statement that a trophoblastic vesicle is present in these forms), and yet some years ago Hubrecht ('04, p. 10) found it difficult "to understand that the name has been misunderstood both by embryologists and gynæcologists." My own feeling is that the more recent developments in his views have tended to obscure rather than to clarify our ideas as to the trophoblast, especially if we must now hold that the chorion or serosa of the Sauropsida is not homologous with that of the Prototheria, which necessarily follows if the extra-embryonal ectoderm of the Sauropsidan is not the same thing as that of the Monotreme.

Assuming that we have formed a correct conception of the trophoblast as a larval membrane, and bearing in mind that it is best developed in the Metatheria and Eutheria, since these alone amongst higher Vertebrates have retained unaltered the viviparous habits of their Protetrapodous ancestors, let us see what basis in fact there is for the statement of Hubrecht ('08, p. 68) that "before the ectoderm and the entoderm have become differentiated from each other there is in mammals a distinct larval cell-layer surrounding (as soon as cleavage of the egg has attained the morula stage) the mother-cells of the embryonic tissues." Now that statement as it stands, I have no hesitation in characterising as entirely

misleading, inasmuch as it is applicable not to the Mammalia as a whole, but, so far as it refers to matters of undisputed fact, to one only of the three mammalian subclasses, viz. the Eutheria. So far as the latter are concerned, practically all observers, as we have seen, are agreed that there is present during at least the early stages of development a complete outer layer of cells which encloses the embryonal anlage or inner cell-mass (that portion of it immediately overlying the latter being termed the "Deckschicht" or "Rauber's layer"). It is, of course, this enveloping layer or trophoblast which Hubrecht interprets as a larval membrane. It fulfils the conditions, and were the Eutheria the only Vertebrates known to us, the idea might be plausible enough.

Turning now to the Metatheria, and remembering that these, according to Hubrecht, are descended from the Eutheria, we should naturally expect to find the supposed larval membrane fully developed, with all its ancestral relations; and so we do if we are content to accept Hubrecht's interpretation of Selenka's results and figures in the case of Didelphys. The "urentodermzelle" of Selenka is for Hubrecht "undoubtedly the mother-cell of the embryonic knob," the ectoderm of Selenka is manifestly the trophoblast—a complete larval layer. It is no doubt unfortunate that Hubrecht had to rely on the work of Selenka as his source of information on the early development of Marsupials, but it must be remembered that he reads his own views into Selenka's figures. On the basis of my own observations on the early ontogeny of Marsupials, I have no hesitation in affirming that a larval membrane, in the sense of Hubrecht, does not exist in any of the forms (Dasyurus, Perameles, Macropus) studied by me. The observations recorded in the preceding pages of this paper demonstrate, in the case of Dasyurus without the possibility of doubt, the entire absence of any cellular layer external to the formative region of the blastocyst, i.e. in a position corresponding to that occupied by Rauber's layer in Eutheria, whilst in the case of Perameles and Macropus, they yield not

the slightest evidence for the existence of any such layer. The formative region of the Marsupial blastocyst, which is undoubtedly the homologue of the inner cell mass of the Eutheria, forms from the first part of the unilaminar blastocyst wall, and is freely exposed. The remainder of the latter is constituted by a layer of non-formative cells, the destiny of which is the same as that of the so-called trophoblast of the Eutheria. I have therefore ventured to suggest that they are one and the same. If, then, the trophoblast is really a larval membrane, we must assume, in the case of the Marsupial, either that its "Deckschicht" portion has been completely suppressed (but why it should have been I fail to understand, unless, perhaps, it is a result of the secondary acquisition by the Marsupials of a shell-membrane, these mammals being even now on the way to secondarily assume the oviparous habit!), or that the non-formative region of the Marsupials is not the homologue of the trophoblast, in which case the Marsupials must be held to have entirely lost the larval membrane, since there is no other layer present which could possibly represent it. These considerations may well give us pause before we calmly accept Hubrecht's conception of the trophoblast as a larval membrane present in all mammals without exception.

Coming now to the Prototheria, we find, according to Hubrecht, "the trophoblastic vesicle . . . yet comparatively distinct," and so it is if we accept the interpretation of Hubrecht of the observations and figures of Semon, Wilson and Hill. The unilaminar blastoderm of these authors is unmistakably the trophoblast. The cells situated internally to that in the region of the white yolk-bed are not entodermal, as suggested by Semon, but constitute for Hubrecht "the mother cells of the embryonic knob." I need only quote again the opinion of Assheton thereanent and express my agreement therewith; he writes ('09, p. 233): "For this view I can see no reason derivable from actual specimens described and figured by those four authors" (Caldwell, Semon, Wilson and Hill). It would appear, then, that the assumption of

Hubrecht of the presence of a larval membrane of the nature postulated in the Prototheria and Metatheria is devoid of foundation in fact, so that there but remains the question of the significance of the outer enveloping layer of the Eutherian blastocyst. As regards that, I venture to think that the alternative interpretation of E. van Beneden and other investigators, which I have attempted to develop in the pages of this paper, affords a simpler and more satisfying explanation of its significance and phylogeny than that advocated by Prof. Hubrecht, an interpretation, moreover, which is more in accordance, not only with all the known facts, but "with sound evolutionary principles" and with the conclusions arrived at by the great majority of comparative anatomists and palæontologists as to the origin and interrelationships of the Mammalia.

And I also venture to think that what has just been said holds true with reference to the views advocated by Mr. Assheton. These views owed their origin to certain appearances which he found in some segmenting ova of the sheep (but, be it noted, not in all those he examined), and he has attempted to re-interpret not only his own earlier observations, but those of other workers on the early ontogeny of the Eutheria in the light of his newer faith, and not only so, he holds that it is also possible to apply that in the interpretation of the early ontogeny of Marsupials (v. '08, p. 235, and '09, p. 229). He maintains that the inner cell-mass of Eutheria is purely ectodermal, and that the enveloping trophoblast layer of the blastocyst arises in common with the entodermal lining of the same and is therefore also entodermal. "On the theory I advocate," he writes ('09, p. 235), "the trophoblast is of Eutherian mammalian origin only and is not homologous to any form of envelope outside the group of Eutherian mammals." These views of Assheton are not only at variance with those of all other investigators who have worked at the early ontogeny of Eutheria, but they are quite irreconcilable with my observations on the development of Dasyurus herein recorded. I claim to have shown in that Marsupial that the formative region, the

homologue of the inner cell-mass, gives origin not only to the embryonal ectoderm, but to the entire entoderm, whilst the non-formative region, whose homology to the trophoblast of Eutheria is admitted by Assheton, arises quite independently of the entoderm and a long time before the latter makes its appearance. There is, then, in Dasyurus no question of a common origin of the entoderm and the non-formative or trophoblastic region of the blastocyst wall. And exception may be taken to Assheton's views on quite other grounds (e. g. the question of the homologies of the fœtal membranes in the series of the Amniota), as he himself is well aware, and as Jenkinson ('00) has also emphasised. I feel, however, I can leave further discussion of Assheton's views until such time as my observations on Dasyurus are shown to be erroneous or inapplicable to other Marsupials.

3. The Entypic Condition of the Eutherian Blastocyst.

If, now, on the basis of the homologies I have ventured to advocate in the preceding pages, we proceed to compare the Metatherian with the Eutherian blastocyst, we have to note that, whereas in the latter the extra-embryonal or trophoblastic ectoderm alone forms the blastocyst wall in early stages and completely encloses the embryonal knot, in the former, the homologous parts, viz. the non-formative or extra-embryonal and the formative or embryonal regions, both enter into the constitution of the unilaminar blastocyst wall, there being no such enclosure of the one by the other as occurs in the Eutherian blastocyst (Text-fig. 2, p. 98). It is characteristic of the Marsupial as of the Monotreme that the embryonal region is from the first superficial and freely exposed. It is spread out as a cellular layer and simply forms part of the blastocyst wall or blastoderm. It is equally characteristic of the Eutherian that the homologous part, the embryonal knot, has at first the form of a compact mass, which is completely enclosed by the trophoblastic ectoderm.

The latter alone constitutes the unilaminar wall of the blastocyst and has the embryonal knot adherent at one spot to its inner surface. The formative cells which compose the knot thus take at first no part in the constitution of the outer wall of the blastocyst, and may or may not do so in later stages according as the covering layer of the trophoblast (the Deckschicht or Rauber's layer) is transitory or permanent. This peculiar developmental condition, characterised by the internal position of the formative or embryonal cells within the blastocyst cavity, has been termed by Selenka ('00) "entypy" (Entypie des Keimfeldes).[1] It is a phenomenon exclusively found in the Eutheria and characteristic of them alone, amongst the mammals. In the Marsupial, as in the Monotreme, the formative cells are freely exposed, and constitute from the first part of the blastocyst wall just as those of the Sauropsida form a part of the general blastoderm. Limited as entypy thus appears to be to the higher mammals, the probability is that we have to do here with a purely secondary, adaptive feature.

If we proceed to inquire what is the significance of this remarkable difference in the early developmental phenomena of the lower and higher mammals, it seems to me that we have to take account, in the first place, of the differences in the structure of their respective eggs, and especially we have to bear in mind that the Eutherian ovum is considerably more specialised than even the Metatherian. It is on the average smaller than the latter, i.e. it has suffered in the course of phylogeny still further reduction in size, and has lost, to an even greater extent than the Marsupial ovum, the store of food-yolk ancestrally present in it. Moreover, it has suffered a still further reduction in respect of its secondary egg-membranes. The Metatherian ovum still retains in its shell-membrane a

[1] "Unter Entypie des Keimfeldes möchte ich daher verstanden wissen: Die nicht durch Bildung typischer Amnionfalten geschehende, sondern durch eine schon während der Gastrulation erfolgende Abschnürung des Keimfeldes ins Innere der Eiblasenhülle (Chorion)" ('00, p. 203).

vestigial representative of the shell of the presumed oviparous common ancestor of the Metatheria and Eutheria. The Eutherian ovum, on the other hand, has lost all trace of the shell in correlation with its more complete adaptation to the conditions of intra-uterine development. The albumen layer is variable in its occurrence, being present in some (e.g. rabbit) and absent in others (e.g. pig, Assheton), whilst the zona itself, though always present, is variable both as to its thickness and the length of time it persists.

Strangely enough, although the prevaling opinion amongst mammalian embryologists is that the Eutherian ovum has been derived phylogenetically from an egg of the same telolecithal and shell-bearing type as is found in the Monotremes, no one, so far as I am aware, has ever taken the shell into account, and ventured to consider in what way its total disappearance from an ovum already greatly reduced in size, might affect the course of the early developmental phenomena. That is what I propose to do here, for in my view it is just in the complete loss of the shell by the Eutherian ovum that we find the key to the explanation of those remarkable differences which are observable between the early ontogeny of the Eutheria and Metatheria, and which culminate in the entypic condition so distinctive of the former. The acquisition of a shell by the Proamniota conditioned the appearance of the amnion. The loss of the shell in the Eutheria conditioned the occurrence in their ontogeny of entypy.

As we have seen, the mammalian ovum, already in the Monotremes greatly reduced in size as compared with that of reptiles, and quite minute in the Metatheria and Eutheria, contains within itself neither the cubic capacity nor the food material necessary for the production of an embryo on the ancestral reptilian lines. We accordingly find that the primary object of the first developmental processes in the mammals has come to be the formation of a vesicle with a complete cellular wall, capable of absorbing nutrient fluid from the maternal uterus and of growing rapidly, so as to provide the space necessary for embryonal differentiation.

In the Monotremes this vesicular stage is rapidly and directly attained as the result, firstly, of the rearrangement of the blastomeres of the cleavage-disc to form a unilaminar blastodermic membrane overlying the solid yolk, and, secondly, of the rapid extension of the peripheral (extra-embryonal) region of the same, in contact with the inner surface of the firm sphere furnished by the egg-envelopes. During the completion of the blastocyst embryonal differentiation remains in abeyance, and practically does not start until after growth of the blastocyst is well initiated.

In the Marsupial, notwithstanding the fact that the ovum has become secondarily holoblastic, the mode of formation of the blastocyst is essentially that of the Monotreme. Cleavage is of the radial type, and owing to the persistence of the shell, which with the zona forms a firm resistant sphere enclosing the egg, the radially arranged blastomeres are able to assume the form of an open ring and to proceed directly to the formation of the unilaminar wall of the blastocyst. The enclosing sphere provides the necessary firm surface over which the products of division of the upper and lower cell-rings of the 16-celled stage can respectively spread towards opposite poles, so as to directly constitute the formative and non-formative regions of the blastocyst wall. In my opinion it is the persistence of the resistant shell-membrane round the ovum which conditions the occurrence in the Marsupial of this direct method of blastocyst formation. As in the Monotreme, so here also embryonal differentiation commences only after the blastocyst has grown considerably in size.

In the Eutheria, on the other hand, in the absence of the shell-membrane, not only is the mode of formation of the blastocyst quite different to that in the Marsupial, but the relations of the constituent parts of the completed structure also differ markedly from those of the homogenous parts in the latter. The cleavage process here leads only indirectly to the formation of the blastocyst, and must be held to be cænogenetically modified as compared with that of

lower mammals. In the cross-shaped arrangement of the blastomeres in the 4-celled stage, in the occurrence of a definite morula-stage and of the entypic condition, we have features in which the early ontogeny of the Eutheria differs fundamentally from that of the Metatheria. They are intimately correlated the one with the other, and are met with in all Eutheria, so far as known, but do not occur either in the Prototheria or the Metatheria, so that we must regard them as secondary features which were acquired by the primitive Eutheria under the influence of some common causal factor or factors, subsequent to their divergence from the ancestral stock common to them and to the Metatheria. Now the cross-shaped 4-celled stage and the morula-stage are undoubtedly to be looked upon simply as cleavage adaptations of prospective significance in regard to the entypic condition, so that the problem reduces itself to this—how came these adaptations to be induced in the first instance? In view of the facts that in the Metatheria, in the presence of the shell-membrane, the formation of the blastocyst is the direct outcome of the cleavage process, and is effected along the old ancestral lines without any enclosure of the formative cells by the non-formative, whilst in the Eutheria, in the absence of the shell-membrane, blastocyst formation results only indirectly from the cleavage-process, is effected in a way quite different from that characteristic of the Metatheria, and involves the complete enclosure of the formative by the non-formative cells, I venture to suggest that the cleavage adaptations which result in the entypic condition were acquired in the first instance as the direct outcome of the total loss by the already greatly reduced Eutherian ovum of the shell-membrane.[1] This view necessarily implies that the presence of a thick zona such as occurs round the ovum in certain Eutheria is secondary, and what we know of this membrane in existing Eutheria is at all events not adverse to that conclusion.

[1] This suggestion I first put forward in a course of lectures on the early ontogeny and placentation of the Mammalia delivered at the University of Sydney in 1904.

Amongst the Marsupials the zona is quite thin (about ·0016 mm. in Dasyurus), presumptive evidence that it was also thin in the ancestral stock from which the Meta- and Eutheria diverged, whilst amongst the Eutheria themselves the zona, as Robinson ('03) has pointed out, is not only of very varying thickness, but persists round the ovum for a very varying period in different species. It appears to be thinnest in the mouse (·001 mm.), in most Eutheria it is considerably thicker (·01 mm., bat, dog, rabbit, deer), whilst in Cavia it reaches a thickness of as much as ·02 mm. In those forms in which the blastocyst early becomes embedded in, or attached to, the mucosa, the zona naturally disappears early. In the rat, mouse and guinea-pig it disappears before the blastocyst is formed. Hubrecht failed to find it in the 2-celled egg of Tupaia, and it was already absent in the 4-celled stage of Macacus nemestrinus, discovered by Selenka and described by Hubrecht. On the other hand, it may persist for a much longer period, up to the time of appearance of the primitive streak (rabbit, dog, ferret). These facts sufficiently demonstrate the variability of the zona in the Eutherian series, and its early disappearance in certain forms before the completion of the blastocyst stage shows that it can have no supporting function in regard to that.

Postulating, then, the disappearance of the shell-membrane and the presence of a relatively thin, non-resistant zona (with perhaps a layer of albumen) round the minute yolk-poor ovum of the primitive Eutherian, and remembering that the ovum starts with certain inherited tendencies, the most immediate and pressing of which is to produce a blastocyst comprising two differentiated groups of cells, the problem is how, in the absence of the old supporting sphere constituted by the egg-envelopes, can such a vesicular stage be most easily and most expeditiously attained? The Eutherian solution as we see it in operation to-day is really a very simple one, and withal a noteworthy instance of adaptation in cleavage (Lillie, '99). In the absence of any firm supporting membrane round the egg, and the consequent impossibility of the blastomeres pro-

ceeding at once to form the blastocyst wall, they are under the necessity of keeping together, and to this end cleavage has become adapted. For the ancestral radial arrangement of the blastomeres in the 4-celled stage, characteristic of the Monotreme and Marsupial, there has been substituted a cross-shaped grouping into two pairs, and, as the outcome of this adaptive alteration in the cleavage planes, there results from the subsequent divisions, not an open cell-ring, as in the Marsupial, but a compact cell-group or morula. In this we again encounter precisely the same differentiation of the blastomeres into two categories, respectively formative (embryonal) and non-formative (trophoblastic) in significance, as is found in the 16-celled stage of the Marsupial, but, since the two groups of cells are here massed together, and in the absence of any firm enclosing sphere, cannot spread independently so as to form directly the wall of the blastocyst, there has arisen the necessity for yet other adaptive modifications. Attention has already been directed to the tardiness of differentiation in the embryonal region of the Monotreme and Marsupial blastocyst, and here in the minute Eutherian morula we find what is, perhaps, to be looked upon as a further adaptive exaggeration of this same feature in the inertness which is at first displayed by the formative cells, and which is in marked contrast with the activity shown by the non-formative ectodermal cells.[1] It is these latter, it

[1] The inertness of the formative cell-mass is accounted for by Assheton ('98, p. 251) as follows: "Now, as the epiblast plays the more prominent part in the formation of the bulk of the embryo during the earliest stages, it clearly would be useless for the embryonic part to exhibit much energy of growth until the old conditions [in particular sufficient room for embryonal differentiation] were to a certain extent regained; hence the lethargy exhibited by the embryonic epiblast in mammals during the first week of development. No feature of the early stages of the mammalian embryo is more striking than this inertness of the embryonic epiblast—or, as I should now prefer to call it, simply epiblast—during the first few days." Assheton, it should be remembered, holds that the inner cell-mass of Eutheria furnishes only the embryonal ectoderm.

should be recollected, which exhibit the greatest growth-energy during the formation of the blastocyst in the Monotreme and Marsupial, and so their greater activity in the Eutherian morula is only what might be expected. Dividing more rapidly than the formative cells, they gradually grow round the latter, and eventually form a complete outer layer enveloping the inert formative cell-group. This process of overgrowth or epiboly is entirely comparable in its effect with the spreading of the extra-embryonal region of the unilaminar blastodermic membrane in the Monotreme to enclose the yolk-mass, and with that of the non-formative cells in the Marsupial to complete the lower hemisphere of the blastocyst, growth round an inert central cell-mass being here substituted for growth over the inner surface of a resistant sphere constituted by the egg-envelopes, such as occurs during the formation of the blastocyst in the Monotreme and Marsupial. Just as the first objective of the cleavage process in the latter is to effect the completion of the cellular wall of the blastocyst, so here the same objective recurs, and is attained in the simplest possible way in the new circumstances, viz. by the rapid envelopment of the formative by the non-formative cells. Thus at the end of the cleavage process in the Eutherian we have formed a solid entypic morula in which an inner mass of formative cells is completely surrounded by an outer enveloping layer of non-formative or tropho-ectodermal cells, homogenous with the extra-embryonal ectoderm of the Sauropsidan and Monotreme and the non-formative region of the unilaminar blastocyst of the Marsupial. Conversion of the solid morula into a hollow blastocyst capable of imbibing fluid from the uterus and of growing rapidly now follows. Intra- or intercellular vacuoles appear below the inner cell-mass, by the confluence of which the blastocyst cavity is established, and the inner cell-mass becomes separated from the enveloping layer of tropho-ectoderm, except over a small area where the two remain in contact.

The complete enclosure of the formative cells of the inner cell-mass by the non-formative ectodermal cells of the

enveloping layer which produces this peculiar entypic condition in the Eutherian blastocyst, I would interpret, then, as a purely adaptive phenomenon, which in the given circumstances effects in the simplest possible way the early completion of the blastocyst wall, and whose origin is to be traced to that reduction in size and in its envelopes which the Eutherian ovum has suffered in the course of phylogeny, in adaptation to the conditions of intra-uterine development. In particular, starting with a shell-bearing ovum, already minute and undergoing its development in utero, I see in the loss of the shell such as has occurred in the Eutheria an intelligible explanation of the first origin of those adaptations which culminate in the condition of entypy. I am therefore wholly unable to accept the view of Hubrecht ('08, p. 78), that "what Selenka has designated by the name of Entypie is—from our point of view—no secondary phenomenon, but one which repeats very primitive features of separation between embryonic ectoderm and larval envelope in invertebrate ancestors."

I see no reason for supposing that the intimate relationship which is early established in many Eutheria between the trophoblastic ectoderm and the uterine mucosa has had anything to do with the origination of the entypic condition. In my view such intimate relationship involving the complete enclosure of the blastocyst in the mucosa only came to be established secondarily, after entypy had become the rule. On the other hand, the peculiar modifications of the entypic condition met with in rodents with "inversion" (e.g. rat, mouse, guinea-pig) are undoubtedly to be correlated, as Van Beneden also believed ('99, p. 332), with the remarkably early and complete enclosure or implantation of the germ in the mucosa such as occurs in these and other Eutheria. Similar views are expressed by Selenka in one of his last contributions to mammalian embryology. He writes ('00, p. 205)—"Dass die Entypie des Keimfeldes und die Blattinversion begünstigt wird durch die frühzeitige Verwachsung der Eiblase mit dem Uterus, ist nicht in Abrede zu stellen. Aber da dieser

Prozess auch in solchen Eiblasen der Säugetiere vorkommen kann, die überhaupt nicht, oder erst später mit dem Uterus verwachsen, so kann die Keimfeld-Entypie zwar durch die frühe Verwachsung veranlasst, aber nicht ausschliesslich hervorgerufen werden." He goes on to remark that—"Die Vorbedingungen zur Entypie müssen in der Struktur der verwachsenden Eiblase gesucht werden," and expresses his agreement with the views of Van Beneden as to the significance to be attributed to the early cleavage phenomena in Eutheria.

The attitude of the illustrious Belgian embryologist whose loss we have so recently to deplore, towards this problem is clearly set forth in the last memoir which issued from his hand. "Je suis de ceux," he wrote ('99, p. 332), "qui pensent que toute l'embryologie des Mammifères placentaires témoigne qu'ils dérivent d'animaux qui, comme les Sauropsides et les Monotrèmes, produisaient des œufs méroblastiques. Je ne puis à aucun point de vue me rallier aux idées contraires formulées et défendues par Hubrecht. L'hypothèse de Hubrecht se heurte à des difficultés morphologiques et physiologiques insurmontables : elle laisse inexpliquée l'existence, chez les Mammifères placentaires, d'une vésicule ombilicale et d'une foule de caractères communs à tous les Amniotes et distinctifs de ces animaux." Holding this view of the origin of the Eutheria, Van Beneden based his interpretation of their early ontogenetic phenomena on the belief that "la reduction progressive du volume de l'œuf d'une part, le fait de son développement intrauterin de l'autre ont du avoir une influence prépondérante sur les premiers processus évolutifs."

Balfour, in his classical treatise, had already some eighteen years earlier expressed precisely the same view. "The features of the development of the placental Mammalia," he wrote ('Mem. Edn.,' vol. iii, p. 289), "receive their most satisfactory explanation on the hypothesis that their ancestors were provided with a large-yolked ovum like that of Sauropsida. The food-yolk must be supposed to have ceased to be developed on the establishment of a maternal nutrition through

the uterus. . . . The embryonic evidence of the common origin of Mammalia and Sauropsida, both as concerns the formation of the layers and of the embryonic membranes is as clear as it can be."

That view of the derivation of the Mammalia receives, I venture to think, striking confirmation from the observations and conclusions set forth in the preceding pages of this memoir, and from it as a basis all attempts at a phylogenetic interpretation of the early ontogenetic phenomena in the Mammalia must, I am convinced, take their origin. Such an attempt I have essayed in the foregoing pages, with what success the reader must judge.

Addendum.

The memoir of Prof. O. Van der Stricht, entitled "La structure de l'œuf des Mammifères (Chauve-souris, Vesperugo noctula): Troisième Partie" ('Mem. de l'Acad. roy. de Belgique,' 2nd ser., t. ii, 1909), came into my hands only after my own paper had reached its final form, and therefore too late for notice in the body of the text. In this extremely valuable contribution, Van der Stricht gives a detailed account of the growth, maturation, fertilisation, and early cleavage-stages of the ovum of Vesperugo, illustrated by a superb series of drawings and photo-micrographs. All I can do here, however, is to direct attention to that section of the paper entitled "Phénomènes de deutoplasmolyse au pôle végétatif de l'œuf" (pp. 92–96), in which the author describes the occurrence in the bat's ovum of just such a process of elimination of surplus deutoplasmic material as I have recorded for Dasyurus. Van der Stricht's interpretation of this phenomenon agrees, I am glad to find, with my own. He writes (pp. 92–93): "Ce deutoplasme rudimentaire, à peine ébauché dans l'ovule des Mammifères, parait être encore trop abondant dans l'œuf de Chauve-souris, car ces materiaux de réserve, en partie inutiles, sont partiellement éliminés, expulsés de la cellule."

To this process of elimination of surplus deutoplasm he applies the name "deutoplasmolyse," and states that "Ce phénomène consiste dans l'apparition de lobules vitellins multiples, en nombre très variable, à la surface du vitellus au niveau du pôle végétatif. Ces bourgeons à peu près tous de même grandeur, les uns étant cependant un peu plus volumineux que les autres, apparaissent dans le voisinage des globules polaires et présentent la structure du deutoplasme. Ils sont formés de vacuoles claires, à l'intérieur desquelles on aperçoit parfois de petits grains vitellins, dont il à été question plus haut. . . . Ce processus de deutoplasmolyse devient manifeste surtout après l'expulsion du second globule polaire, pendant la période de la fécondation. Il peut être très accentué, au stade du premier fuseau de segmentation et au début de la segmentation de l'œuf, notamment sur des ovules divisés en deux et en quatre (figs. 59, 61, 62, d)." It would therefore appear that, whilst in Dasyurus the surplus deutoplasm is eliminated always prior to the completion of the first cleavage and in the form of a single relatively large spherical mass, in Vesperugo it is cast off generally, though not invariably, before cleavage begins, and in the form of a number of small separate lobules.

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EXPLANATION OF PLATES 1–9,

Illustrating Prof. J. P. Hill's paper on "The Early Development of the Marsupialia, with Special Reference to the Native Cat (Dasyurus viverrinus)."

[All figures are from specimens of Dasyurus, unless otherwise indicated. Drawings were executed with the aid of Zeiss's camera lucida, except figs. 61–63, which were drawn from photographs.]

List of Common Reference Letters.

Abn. Abnormal blastomere, fig. 37. *alb.* Albumen. *cg.* Coagulum. *d. p.* Discus proligerus. *d. z.* Deutoplasmic zone. *emb. a.* Embryonal area. *emb. ect.* Embryonal ectoderm. *ent.* Entoderm. *f. ep.* Follicular epithelium. *f. a.* Formative area of blastocyst wall. *f. c.* Formative cell. *f. z.* Formative zone. *i. c.* Internal cell, fig. 34. *l. ent.* Limit of extension of entoderm. *l. p.* Incomplete area of blastocyst wall at lower pole. *p. b*^{1}. First polar body. *p. b*^{1}. *s.* First polar spindle. *p. b*^{2}. *s.* Second polar spindle. *p. s.* Perivitelline space. *s. m.* Shell-membrane. *sp.* Sperm in albumen. *tr. ect.* Non-formative or trophoblastic ectoderm (tropho-ectoderm). *y. b.* Yolk-body. *z. p.* Zona.

PLATE 1.

Fig. 1.—Photo-micrograph (× 150 diameters) of the full-grown ovarian ovum, ·27 × ·26 mm. diameter. The central deutoplasmic zone (*d. z.*) and the peripheral formative zone (*f. z.*), in which the

vesicular nucleus (·05 × ·03 mm. diameter) is situated, are clearly distinguishable. The zona (*z. p.*) measures ·0021–·0025 mm. in thickness. Outside it are the follicular epithelial cells of the discus proligerus (*d. p.*), which is thickened on the upper side of the figure, where it becomes continuous with the membrana granulosa. (D. v i v., 21 . vii . '04, $\frac{1}{2}$. Hermann's fluid and iron-hæmatoxylin.)

Fig. 2.—Photo-micrograph (× 150) of ripe ovarian ovum (in which first polar body is separated and second polar spindle is present, though neither is visible in figure), ·29 × ·23 mm. maximum diameter. Follicle 1·4 × 1·1 mm. diameter. The ovum exhibits an obvious polarity. Deutoplasmic zone (*d. z.*) in upper hemisphere; formative zone (*f. z.*) forming lower. (D. v i v., 14, 26 . vii . '02, $\frac{8}{2-6}$. Flemming's fluid and iron-hæmatoxylin.)

Fig. 3.—Photo-micrograph (× 150) of ripe ovarian ovum (·28 × ·24 mm. diameter) with first polar body (*p. b*^{1}.) and second polar spindle. First polar body, ·026–·03 × ·01 mm. Second polar spindle, ·013 mm. in length. (D. v i v., 14, 26 . vii . '02, $\frac{10}{4-3}$. Flemming's fluid and iron-hæmatoxylin.)

Fig. 4.—Photo-micrograph (× 256) of ovarian ovum in process of growth ("pseudo-alveolar" stage). Ovum, ·26 × ·20 mm. diameter. Zona, ·0017–·002 mm. in thickness. (D. v i v., 14, 26 . vii . '02, $\frac{2}{3-1}$. Hermann, iron-hæmatoxylin.)

Fig. 5.—Photo-micrograph (× 1250) of peripheral region of ripe ovarian ovum (·28 × ·126 mm. diameter) with first polar spindle (·015 × ·013 mm.). (D. v i v., 23 . vii . '02, $\frac{7}{2-8}$. Ohlmacher's fluid, iron-hæmatoxylin.)

Fig. 6.—Photo-micrograph (× 1250) of peripheral region of ripe ovarian ovum (·26 × ·18 mm.), showing first polar body (*p. b*^{1}.) (·03 × ·006 mm.). (D. v i v., 14, 26 . vii . '02, $\frac{4}{1-V}$. Flemming, iron-hæmatoxylin.)

Fig. 7.—Photomicrograph (× 1250) of peripheral region of ovum, fig. 3, showing portion of first polar body (*p. b*^{1}.), and the second polar spindle. The dark body lying between *p. b*^{1}. and the surface of the ovum is a displaced red blood-corpuscle.

Figs. 8 and 9.—Photo-micrographs (× about 84) of unsegmented ova, respectively ·33 mm. and ·35 mm. in diameter, from the uterus, taken immediately after their transference to the fixing fluid (picro-nitro-osmic acid), showing the shell-membrane (*s. m.*), laminated albumen (*alb.*), with sperms (*sp.*), the zona (*z. p.*), perivitelline space (*p. s.*), and the body of the ovum, with its formative (*f. z.*), and deutoplasmic (*d. z.*) zones. (D. v i v., 15, 19 . vii . '01.)

Fig. 10.—Photo-micrograph (× 150) of section of unsegmented ovum almost immediately after its passage into the uterus, showing the very

thin shell-membrane externally (*s. m.*) (about ·0016 mm. in thickness), the albumen (*alb.*), zona (*z. p.*), and the deutoplasmic (*d. z.*) and formative (*f. z.*) zones of its cytoplasmic body. The male pronucleus is visible in the formative zone. Diameter of entire egg about ·29 mm. (D. viv., 15, 19 . vii . '01, $\frac{5}{3}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 11.—Photo-micrograph (× 150) of section of unsegmented ovum from the uterus, slightly older than that of fig. 10. Diameter of entire egg in fresh state ·34–·35 mm., of the ovum proper ·3 × ·28 mm.; thickness of shell, ·0024 mm. In the figure the female pronucleus is visible near the centre of the formative zone (*f. z.*), and the male pronucleus lies a little above it and to the right. The perivitelline space (*p. s.*) is partially occupied by coagulum. (D. viv., 21 . v . '03, $\frac{7}{2}$. Hermann, iron-hæmatoxylin.)

PLATE 2.

Fig. 12.—Photo-micrograph (× 150) of an unsegmented ovum from the uterus, of the same batch as that of fig. 11, and ·34 mm. in diameter. The two pronuclei are visible in the central region of the formative zone.

Fig. 13.—Photo-micrograph (× 330) of uterine ovum. Stage of first cleavage spindle. Diameter, ·315 mm. (D. viv., 1, 15 . vii . '01, $\frac{8}{2}$. Picro-nitro-osmic, iron-hæmatoxylin.)

Fig. 14.—Photo-micrograph (× about 78) of egg in the 2-celled stage, taken immediately after its transference to the fixing fluid. Lateral view. *y. b.* Yolk body. Diameter of entire egg about ·34 mm. (D. viv., 1, 15 . vii . '01. Picro-nitro-osmic.)

Fig. 15.—Photo-micrograph (× about 78) of another 2-celled egg, seen from lower pole. Diameter, ·35 mm. (D. viv., 4 B, 23 . vi . '02. Perenyi's fluid.)

Fig. 16.—Photo-micrograph (× about 78) of another 2-celled egg, of the same batch as preceding. End view, showing one of the two blastomeres and the yolk-body (*y. b.*).

Fig. 17.—Photo-micrograph (× 150) of vertical section of 2-celled egg, ·34 mm. in diameter, showing the shell-membrane (·0064 mm. thick), traces only of the albumen, the zona (*z. p.*), and the two blastomeres (the left one measuring, from the sections, ·16 × ·18 × ·10 mm., its nucleus ·031 × ·027 mm.; the right one, ·16 × ·19 × ·09 mm., its nucleus, ·03 × ·028 mm.). Note the differentiation in their cytoplasmic bodies. (D. viv., 6, 21 . vii . '01, $\frac{6}{2}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 18.—Photo-micrograph (× 150) of vertical section of 2-celled egg, ·32 mm. in diameter, with shell-membrane ·005 mm. thick, showing the two blastomeres, and enclosed between their upper ends the yolk-

body (*y. b.*). (D. viv., 1, 15 . vii . '01, $\frac{2}{2}$. Picro-nitro-osmic, iron-hæmatoxylin.)

Figs. 19 and 20.—Photo-micrographs (× about 70) of 4-celled eggs taken immediately after transference to Perenyi's fluid. Fig. 19, side view, showing yolk-body (*y. b.*); fig. 20, polar view. Diameter of entire egg about ·35 mm. (D. viv., 14 B, 18 . vi . '02. Perenyi.)

Fig. 21.—Photo-micrograph (× about 70) of another 4-celled egg, from the same batch as the preceding, seen from lower pole.

Fig. 22.—Photo-micrograph (× 150) of section of 4-celled egg of same batch as those of figs. 19 and 20. The two right and the two left blastomeres respectively form pairs, so that the plane of the first cleavage is parallel with the sides of the plate, that of the second with the top and bottom of the same. The two left blastomeres are still connected by a narrow cytoplasmic bridge. Thickness of shell, ·0072 mm.

Fig. 23.—Photo-micrograph (× 150) of a vertical section through a 4-celled egg, ·35 mm. in diameter, showing two of the blastomeres and a small portion of the yolk-body (*y. b.*). Note, as in fig. 22, the marked differentiation in the cytoplasm of the blastomeres. (D. viv., 4, 27 . vi . '01. Picro-nitro-osmic, iron-hæmatoxylin.)

Figs. 24 and 25.—Photo-micrographs (× 140) of horizontal sections through a 16-celled egg, ·38 mm. diameter, fig. 24 showing the eight larger, more yolk-rich cells of the lower (non-formative) ring, and fig. 25 the eight smaller, less yolk-rich cells of the upper (formative) ring. Shell ·0075 mm. in thickness, yolk-body (not included in the figures) ·11 × ·10 mm. in diameter. (D. viv., 3 B, 26 . vi . '01; 15, $\frac{5}{3}$ and $\frac{6}{4}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 26.—Photo-micrograph (× 140) of a vertical section of an egg of the same batch and size as that represented in figs. 24 and 25, but with seventeen cells—formative = 9 (6 + [1 × 2] + 1) in division; non-formative = 8. Two of the formative cells (*f. c.*) of the upper ring are seen enclosing between them the faintly marked yolk-body (*y. b.*), and below them two of the much more opaque non-formative cells (*tr. ect.*) of the lower ring.

PLATE 3.

Fig. 27.—Photo-micrograph (× about 76) of the just completed blastocyst, ·39 mm. in diameter. From a spirit specimen. The dark spherical mass (*cg.*) in the blastocyst cavity is simply coagulum, produced by the action of the fixative (picro-nitro-osmic acid) on the albuminous fluid which fills the blastocyst cavity. (D. viv., 2 B, 16 . vii . '01.)

Fig. 28.—Photo-micrograph (× about 76) of a blastocyst of the same batch as the preceding, ·45 mm. in diameter. From a spirit specimen. *cg.* Coagulum.

Fig. 29.—Photo-micrograph (× about 75) of another blastocyst, ·45 mm. diameter, of the same batch as the preceding, but taken immediately after transference to the fixative. Viewed from the upper pole. *y. b.* Yolk-body seen through the unilaminar wall.

Fig. 30.—Photo-micrograph (× about 75) of a blastocyst of the same batch as the preceding, about ·39 mm. in diameter, in which the cellular wall has not yet been completed over the lower polar region.

Fig. 31.—Photo-micrograph (× 140) of a section of a blastocyst, ·39 mm. diameter, of the same batch as the preceding and at precisely the same developmental stage, the cellular wall having yet to be completed over the lower polar region (*l. p.*). In the blastocyst cavity is seen the yolk-body (*y. b.*) partially surrounded by a mass of coagulum (*cg.*). (D. viv., 2 B, 16 . vii . '01, m. = ·39, $\frac{7}{3}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 32.—Photo-micrograph (× 140) of another blastocyst, ·41 mm. in diameter, of the same batch as the preceding, also with the cellular wall still absent over the lower polar region. Shell-membrane ·0075 mm. in thickness. *y. b.* Yolk-body. *c. g.* Coagulum. The cellular wall comprises about 130 cells.

Fig. 33.—Photo-micrograph (× 140) of a blastocyst of the same batch as the preceding, with a complete unilaminar cellular wall. *y. b.* Yolk-body, in contact with inner surface of wall, in the region of the upper pole.

Fig. 34.—Photo-micrograph (× 100) of a section of a blastocyst ·57 mm. in diameter. *i. c.* Internal cell. (D. viv., 29 . vi . '04, $\frac{1.5}{2}$. Picro-nitro-osmic.)

Fig. 35.—Photo-micrograph (× 100) of a section of a blastocyst, ·73 mm. diameter, of the same batch as the preceding, shell, ·0045 mm. thick.

Fig. 36.—Photo-micrograph (× 100) of a section of a blastocyst ·66 mm. diameter, of the same batch as the preceding. Lower hemisphere opposite yolk-body (*y. b.*) formed of larger cells than upper. Hermann fixation.

Fig. 37.—Photo-micrograph (× 140) of section of an abnormal vesicle, ·397 mm. diameter of the same batch as the normal vesicles represented in figs. 27–33. *abn.* large binucleate cell, regarded as a blastomere of the lower hemisphere which has failed to divide in normal fashion, cf. text, p. 42.

PLATE 4.

Fig. 38.—Photo-micrograph (× 10) of entire blastocyst 4·5 mm. diameter to show the junctional line (*j. l.*) between formative and non-formative regions. From a spirit specimen. (D. viv., *β*, 25 . vii . '01. Picro-nitro-osmic.)

Fig. 39.—Photo-micrograph (× about 10) of an entire blastocyst, 4·5 mm. diameter with distinct embryonal area (*emb. a.*). (D. viv., 5, 18 . vii . '01.)

Fig. 40.—Photo-micrograph (× 10) of entire blastocyst about 5 mm. diameter showing embryonal area (*emb. a.*), peripheral limit of entoderm (*l. ent.*), and the still unilaminar region of the wall (*tr. ect.*). (D. viv., 8 . vi . '01.)

Fig. 41.—Photo-micrograph (× 150) of an in toto preparation of the wall of a blastocyst of 3·5 mm. diameter. (D. viv., 16, 21 . vii . '01.)

Fig. 42.—Photo-micrograph (× 150) of an in toto preparation of the wall of a blastocyst of 3·25 mm. diameter. *j. l.* Junctional line between the formative (*f. a.*) and non-formative (*tr. ect.*) regions of the wall. (D. viv., 24 . vii . '01.)

Figs. 43 and 44.—Photo-micrographs (× 150) of in toto preparations of the wall of 4·5 mm. blastocyst showing the junctional line between the formative (*f. a.*) and non-formative (*tr. ect.*) regions. (D. viv., *β*, 25 . vii . '01. Picro-nitro-osmic and Ehrlich's hæmatoxylin.)

Fig. 45.—Photo-micrograph (× 150) of a corresponding preparation of the wall of a more advanced 4·5 mm. blastocyst ('99 stage), in which the two regions of the wall are now clearly distinguishable. (D. viv., 8 . 7 . '99. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

Fig. 46.—Photo-micrograph (× 150) of a corresponding preparation of a slightly more advanced blastocyst ('04 stage). (D. viv., 6 . 7 . '04. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

PLATE 5.

Fig. 47.—Photo-micrograph (× 150) of an in toto preparation of the formative region of a 6 . 7 . '04 blastocyst, showing the proliferation of spherical internal cells referred to in the text, p. 53.

Fig. 48.—Photo-micrograph (× 150) of an in toto preparation of the wall of a vesicle of the same batch as that represented in fig. 39, in which a small part of the junctional line between the embryonal ectoderm and the extra-embryonal (*tr. ect.*) is visible, the free edge of the entoderm (*ent.*) not having reached it. (D. viv., 5, 18 . vii . '01. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

Fig. 49.—Photo-micrograph (× 150) of a corresponding preparation of a vesicle of the same batch as the preceding, in which the wavy and irregularly thickened free edge of the entoderm (*ent.*) practically coincides with the junctional line and so conceals it from view.

Fig. 50.—Photo-micrograph (× 150) of an in toto preparation of a vesicle (8 . vi . '01 batch) viewed from the inner surface as in the corresponding preceding figures. The entoderm in the region of the embryonal area has been removed, so that one sees the inner surface of the embryonal ectoderm (*emb. ect.*); it is still in situ, though not in a quite intact condition over the adjoining portion of extra-embryonal ectoderm. The entoderm has not yet extended over the region indicated by the reference line to *tr. ect.*, so that here the extra-embryonal ectoderm is clearly visible. The junctional line is apparent. (D. viv., 8 . vi . '01. Picro-nitro-osmic. Ehrlich's hæmatoxylin.)

Fig. 51 (Plate 3).—Photo-micrograph (× 310) of a section of a 30-celled egg of Perameles obesula; egg *b*. ·24 × ·23 mm. diameter, showing the unilaminar layer formed by the blastomeres.

Fig. 52 (Plate 3).—Photo-micrograph (× 240) of a section of a blastocyst of P. nasuta ·29 × ·26 mm. diameter, showing the shell-membrane (*s.m.*), zona (*z.p.*), and the unilaminar cellular wall. The portion of the latter adjacent to the reference lines is composed of smaller but thicker cells than the remainder.

PLATE 6.

Figs. 53 and 54.—Drawings (× 84) of a 6-celled egg ·34 mm. diameter, fig. 53 showing a side view and fig. 54 a view from the lower pole. Observe the characteristic ring-shaped arrangement of the blastomeres. *y. b.* Yolk-body, the shell-membrane, albumen layer with sperms included, and the zona are readily distinguishable. Outlines drawn with the aid of the camera lucida immediately after transference of the egg to the fixing fluid. (D. viv., 22. 16 . vii . '01.)

Figs. 55 and 56.—Drawings (× about 88) of a 16-celled egg (about ·37 mm. diameter) as seen from the side and lower pole respectively, from the same batch as the eggs represented in figs. 24, 25, and 26. The characteristic arrangement of the blastomeres in two superimposed, open rings (each of eight cells) and the difference in size between the cells of the two rings are evident. The irregular body (*c.g.*) seen in the cleavage cavity in fig. 56 is a mass of coagulum. Drawn from a spirit specimen. The albumen layer as represented in fig. 56 is too thick. (D. viv., 3 B, 26 . vi . '01.)

Figs. 57 and 58.—Drawings (× about 85) of a 12-celled egg (·38 mm. diameter) as seen from the side and lower pole respectively. Four of

the blastomeres of the 8-celled stage have already divided $(4 + 4 \times 2) = 12$. From a spirit specimen and from same batch as preceding.

Fig. 59.—Drawing (× about 88) of a 31-celled egg (·375 mm. diameter) as seen from the lower pole. From a spirit specimen and from the same batch as the preceding. The irregular body in the blastocyst cavity is formed by coagulum. Formative cells = 16; non-formative = 14 + 1 in division.

Fig. 60.—Drawing (× about 88) of another 31-celled egg (·375 diameter) from the same batch as the preceding. Side view.

Fig. 61.—Drawing (× 100) of an entire blastocyst (·39 mm. diameter) from the same batch as those shown in figs. 27–29.

Fig. 62.—Drawing (× about 80) of an entire blastocyst (·4 mm. diameter) from the same batch as the preceding.

Fig. 63.—Drawing (× 80 of an entire blastocyst (·6 mm. diameter) made from a photograph taken directly after transference of the specimen to the fixing fluid. Cells of lower hemisphere with much more marked perinuclear areas of dense cytoplasm than those of the upper. D. viv., 2, 11 . vii . '01.)

Fig. 64.—Section of the wall of a blastocyst, 2·4 mm. diameter (× 630). (D. viv., 7 . vi . '01.)

Figs. 65, 66, 67.—Drawings (× 630) of small portions of in toto preparations of the formative region of 6 . 7 . '04 blastocysts to demonstrate the mode of origin of the primitive entodermal cells (*ent.*, fig. 67). Fig. 65 shows a dividing entodermal mother-cell in position in the unilaminar wall, surrounded by larger lighter staining cells (prospective embryonal ectodermal cells). In fig. 66 is seen a corresponding cell, a portion of whose cell-body has extended inwards so as to underlie (overlie in figure) one of the ectodermal cells of the wall. In fig. 67 are seen two entodermal cells, evidently sister-cells, the products of the division of such a cell as is seen in figs. 65 or 66. One of them (the upper) is still a constituent of the unilaminar wall, the other (*ent.*) is a primitive entodermal cell, definitely internal. (D. viv., 6 . 7 . '04. Picro-nitro-osmic. Ehrlich's hæmatoxylin.)

PLATE 7.

Figs. 68, 69, 70.—Drawings (× 630) of portions of preparations similar to the above. For description see text. (D. viv., 6, 7, '04.)

Fig. 71.—Drawing (× about 630) of a portion of an in toto preparation of the formative region of an '01 blastocyst showing two primitive entodermal cells, one of them in division. (D. viv., β, 25 . vii . '01. Picro-nitro-osmic and Ehrlich.)

Fig. 72.—Drawing (× 630) corresponding to the above, from the formative region of a 6 . 7 . '04 blastocyst, also showing two primitive entodermal cells, evidently sister-cells.

PLATE 8.

Figs. 73, 74, 76.—Sections of the formative region of 6 . 7 . '04 blastocysts, showing the attenuated shell-membrane, the unilaminar wall, and in close contact with the inner surface of the latter, the primitive entodermal cells (*ent.*) (× 630).

Fig. 75.—Section corresponding to the above, showing an entodermal mother-cell (*ent.*), part of whose cell-body underlies the adjacent ectodermal cell of the wall. The spheroidal inwardly projecting cell on the left is probably also an entodermal mother-cell (× 630).

Fig. 77.—Section (× 630) of the non-formative region of a 6 . 7 . '04 blastocyst.

Fig. 78.—Section (× 630) of the embryonal area, and the adjoining portion of the still unilaminar extra-embryonal region of a blastocyst of the 5 . '01 stage. *emb. ect.* Embryonal ectoderm. *ent.* Entoderm. *tr. ect.* Extra-embryonal ectoderm (tropho-ectoderm). The position of the junctional line is readily recognisable. (D. viv., 5, 18 . vii . '01. Picro-nitro-osmic and Delafield's hæmatoxylin.)

Fig. 79.—Section (× 630) through the corresponding regions in an 8 . vi . '01 blastocyst. Note the thickening of the embryonal ectoderm (*emb. ect.*), and the peripheral extension of the entoderm (*ent.*) below the tropho-ectoderm. (D. viv., 8 . vi . '01. Picro-nitro-osmic and Delafield.)

Fig. 80.—Section (× 600) through the formative (embryonal) region of a blastocyst of P. nasuta, 1·3 mm. in diameter. It is thicker than that of the Dasyure blastocyst at the corresponding stage of development; the primitive entodermal cells are well marked.

Fig. 81.—Section (× 600) corresponding to the above from another 1·3 mm. blastocyst of P. nasuta, of the same batch as the preceding, but apparently very slightly earlier, the entodermal cells being still in process of separating from the unilaminar wall. *ent.* Entoderm. *tr. ect.* Tropho-ectoderm.

PLATE 9.

Fig. 82.—Section (× about 430) of a section of a blastocyst of M. ruficollis ·35 mm. in diameter, showing the major portion of the formative region (*f. a.*) and a small portion of the non-formative (*tr. ect.*).

The shell-membrane varies in thickness in the sections from ·005 mm. over the former region to ·003 mm. over the latter.

Figs. 83, 84, 85.—Drawings (× 630) of small portions of the formative (and in fig. 83 of the adjoining portion of the non-formative) region of the above blastocyst of M. ruficollis more highly magnified. *ent.* Primitive entodermal cells. Note in fig. 83 a cell of the wall in division, the axis of the spindle being oblique to the surface.